STUDIES ON SAPROPHYTIC MYCOBACTERIA AND CORYNEBACTERIA.

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(Plates i–ii; seven Text-figures.)

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Introduction.

The genera *Mycobacterium* and *Corynebacterium* were introduced by Lehmann and Neumann in 1896 and had as type species, respectively, the tubercle bacillus, *Myc. tuberculosis*, and the diphtheria bacillus, *Cor. diphtheriae*, two important pathogens which, together with a few related forms, distinguished themselves from all other bacteria known at that period by their characteristic morphology. Since then, numerous saprophytic and parasitic species have been added to the two genera.

In Lehmann and Neumann's classical definition, *Mycobacterium* was distinguished by its formation of slender, frequently branched rods of irregular length, after staining with hot carbol-fuchsin not easily decolorized by treatment with mineral acids ("acid-fast"), and *Corynebacterium* by its tendency to form cells of irregular thickness, club- or wedge-shaped, sometimes branched, not acid-fast, but often showing an irregular bar- or belt-staining.

Because of the branching, Lehmann and Neumann regarded the two genera as closely related to the actinomycetes, from which they are distinguished by the lack of a typical mycelium (cf. Miehe, 1909; Haag, 1927).

Lehmann and Neumann's definitions have in all essential points been adopted in subsequent treatises upon these organisms, for instance, Andrews, Bullock *et al.* (1923), Bergey (1923–30), Haag (1927), but the distinction between the two genera is by no means simple, as the following survey of their general morphology and biology will show.

1. *Cell Morphology.*—The names *Corynebacterium* and *Mycobacterium* were designed to indicate a prevalence of club-shaped cells in the former genus, and a tendency to branching in the latter. It is generally conceded that the typical clubs are as a rule, but not constantly, present in *Cor. diphtheriae*, but mostly absent in the non-virulent "diphtheroids" (Andrews, Bullock *et al.*, 1923; Gins and Fortner, 1926; Kliewe, 1927). In older cultures of corynebacteria there is often an abundance of very big, spherical to lemon-shaped cells almost as big as yeast cells (Dale, 1910; de Negri, 1916; Bergstrand, 1918–23; Mellon, 1920–26; Brown and Orcutt, 1920; Grasset and Grasset, 1930), or smaller round cells appearing like true cocci (Madsen, 1896; Mellon, 1917–26; de Negri, 1916; Walker and Adkinson, 1917; Andrews, Bullock *et al.*, 1923); these coccoid forms can be made entirely predominant by special methods of cultivation (Smirnow, 1908; Malchereck, 1932; Pope and Pinfield, 1932). Mycobacteria do not produce clubs in young, but sometimes in old cultures (Metschnikoff, 1888; Rabinowitsch, 1897;

Korn, 1899; Tobler, 1901; Büttner, 1926; Haag, 1927); really big "cystites", as in the corynebacteria, seem only to have been observed in media containing KJ (Péju and Rajat, 1907). Coccoid cells may be formed in truly acid-fast saprophytic mycobacteria (Moeller, 1898; Korn, 1899; Söhngen, 1913; Ørskov, 1923) as well as in less acid-fast forms approaching the genus *Proactinomyces* (Gray and Thornton, 1928; Jensen, 1931–32). Branching is mostly an occasional phenomenon in corynebacteria (Bernheim and Folger, 1896; Hill, 1902; Abbott and Gildersleeve, 1904), although it may be very prevalent in certain strains of *Cor. diphtheriae* under special conditions of growth, such as media containing lithium chloride (Maassen, 1904), or reduced oxygen pressure (Martin *et al.*, 1924). Some authors (Enderlein, 1925; Kuhn and Sternberg, 1931) even deny the existence of branching in the said species. Also in the mycobacteria branching may be infrequent (Seiffert, 1932) or quite absent (Kuhn and Sternberg, 1931).

2. *Staining Properties.*—As Lehmann and Neumann (1920–27) point out, the mycobacteria are not always strongly acid-fast. Forms only weakly or inconstantly acid-fast were described by Olschanetzsky (1902), Bertani (1913), Ørskov and Jensen (1926), Büttner (1926), Haag (1927) and Eichbaum (1932); see also Gray and Thornton (1928) and Jensen (1931). The very type species, *Myc. tuberculosis*, is not acid-fast in quite young cultures (Krylow, 1912; Wherry, 1913). The same is true to a still higher degree of *Myc. phlei* (Cantacuzène, 1905). The acid-fastness may be lost, temporarily or even permanently, by special methods of cultivation, such as acid media, growth in mixture with other microorganisms, etc. (Frei and Pockschischewsky, 1910; Wherry, 1913; Thompson and O'Brien, 1920; Vaudremer, 1921; Machado, 1927; Schachschuwarly and Woldrich, 1929; Eberson and Sweeney, 1931; Kirchner *et al.*, 1930). Some corynebacteria, on the other hand, show some acid-fastness (Wolbach and Honeij, 1915; Bergstrand, 1918; Haag, 1927; Daines and Austin, 1932; Martinaglia, 1932; Knorr, 1932). Haag even found certain strains of *Cor. diphtheriae* as acid-fast as the weakly acid-fast *Myc. eos.* The mycobacteria have always been reported as grampositive, although they may be gramnegative while quite young (Krylow, 1912). The same applies to the corynebacteria, although some of them are but weakly grampositive. The so-called metachromatic granules, which are characteristic of the diphtheria bacillus, occur very inconstantly among other corynebacteria (Kliewe, 1927; Schroeder, 1931) and therefore afford no differentiation between the two genera.

3. *Motility* has sometimes been alleged in mycobacteria (Ferran, 1897; Schumowski, 1898; Moeller, 1899; Tobler, 1901; Courmont and Descos, 1902; Hawthorn, 1903), but as flagella have never been demonstrated, these statements might seem due to observational errors. Ferran, indeed, claims to have stained flagella, but his alleged transformation of *Myc. tuberculosis* is of such a character that one cannot but suspect a contamination. No doubtless corynebacteria have been found motile.

4. *Mode of Cell Division.*—Kurth (1898) first described the characteristic process of cell division in *Cor. diphtheriae*: a cell grows to a certain length, a line of division is formed at the middle, and the daughter cells bend suddenly into an angle, thereby producing the V- or L-shaped figures so eminently characteristic of microscopical preparations of these organisms. This phenomenon was later termed "snapping" growth by Hill (1902), and shown by Graham-Smith (1910) to be characteristic of all diphtheroids. Bergey (1923–30) has included it in the diagnosis of *Corynebacterium.* On this basis, too, did Kisskalt and Berend (1918) transfer several organisms, previously known as *Bacterium*, to *Coryne-*

bacterium. Ørskov found this mode of division, which he termed "angular growth", perfectly constant in mycobacteria, corynebacteria and certain actinomycetes. A different type of division was observed in *Myc. tuberculosis* by Miehe (1909): after division, the ends of the daughter cells bend, slip past each other, and grow into parallel bundles (the "slipping growth" of Graham-Smith, 1910). Similar observations were made by von Faber (1912) on quite different mycobacteria. Miehe's observations were confirmed and extended by Ørskov (1923–32), Haag (1927), and Gardner (1929), who showed that the growth starts with the "snapping" type, which is later superseded by the "slipping". Georgevitch (1916) has, independently of these authors, beautifully demonstrated both slipping and angular growth in an organism from the leaf nodules of *Pavetta* (cf. von Faber, 1912). A certain unequal division, resembling the budding of yeasts, has been observed in diphtheroids by Bergstrand (1918–19) and Mellon (1920); the latter author gave this phenomenon the somewhat bizarre name of "lapolar gemmation". It represents probably a breaking-off of side-branches (cf. Hill, 1902).

5. *Fermentative Reactions.*—The mycobacteria seem as a whole incapable of decomposing carbon-compounds with the formation of organic acids, according to Haag (1927), and particularly to the very careful studies of Merrill (1930–31). A slight formation of acetic acid was reported by Söhngen (1913); also Birch-Hirschfeld (1932) and Eichbaum (1932) mention a slight acid-formation (see also Thomson, 1932). There is a single instance recorded (Kersten, 1909) of an acid-fast bacterium forming gas in sugar-media; since no details were given, this isolated case may be regarded with scepticism. Most corynebacteria ferment sugars and related compounds with the formation of acids, but not of gas, and an enormous amount of work has been devoted to the classification of corynebacteria on the basis of these reactions (for references, see Andrews, Bullock *et al.*, 1923; Kliewe, 1927; Schroeder, 1931). These studies have shown that all corynebacteria with the exception of one group, known as "Hofmann's bacillus" or *Cor. pseudodiphtheriticum*, are able to produce acid from some carbohydrate or other.

6. *Nitrogen Requirements.*—Most mycobacteria are not fastidious in their requirements for nitrogenous food. Proskauer and Beck (1894) showed that simple amino-acids and ammonium-salts, but not nitrate, would serve as nitrogenous food for *Myc. tuberculosis*; this was confirmed by Kondo (1925) and Merrill (1931). Saprophytic mycobacteria grow well with nitrate (Haag, 1927). The diphtheria bacillus and the diphtheroids will, as a rule, demand a rather rich medium containing protein. Haag (1927) considered this a feature of distinction between mycobacteria and corynebacteria, but the difference is not absolute, since *Cor. diphtheriae* will grow in protein-free media under certain conditions (Braun and Mündel, 1929).

7. *Proteolysis.*—No mycobacteria are capable of liquefying gelatin or digesting milk. Dernby and Näslund (1922) found *Myc. tuberculosis* devoid of proteolytic ectoenzyme, but possibly containing small amounts of proteolytic endoenzyme (cf. Corper and Sweany, 1918); it is also capable of forming ammonia from peptone, probably through the action of endoenzymes (Merrill, 1930). *Cor. diphtheriae* does not liquefy gelatin or digest milk (Eijkmann, 1901), but possesses proteolytic endoenzymes (Dernby and Siewe, 1923). Those few authors who have studied the growth of diphtheroids in milk (v. Przewoski, 1912; Belenky and Popowa, 1930; Steck, 1932) do not report any proteolysis in this medium, but a few gelatin- or serum-liquefying diphtheroids have been described by Müller (1908), Mellon

(1917), Eberson (1918), Barratt (1924) and Süssmann (1928). Also *Cor. pyogenes* (Brown and Orcutt, 1920) is proteolytic, as well as some of the saprophytic corynebacteria of Kisskalt and Berend (1918) and some alleged variants of *Cor. diphtheriae* (Maver, 1931).

8. *Utilization of Paraffine.*—Söhngen (1913), Büttner (1926), Haag (1927) and Jensen (1932) have shown the ability of saprophytic mycobacteria and several actinomycetes to use paraffines as source of energy. Haag considered this a decisive point in the differentiation between mycobacteria and corynebacteria, which latter do not grow on paraffine.

9. *Oxygen Requirements.*—The tubercle bacillus is strictly aerobic, as well as most saprophytic mycobacteria. Acid-fast bacteria claimed to be facultative anaerobes were mentioned by Korn (1899), Karlinski (1901), and Olschanetzsky (1902), but since no technical details were given, this cannot be regarded as proved. Among the corynebacteria we find strictly aerobic, facultative anaerobic, and microaerophilic forms. *Cor. diphtheriae* will grow under more or less anaerobic conditions, although, according to Pesch and Gottschalck (1924), not in entirely oxygen-free atmosphere, whereas most diphtheroids are decidedly aerobic (Pesch and Gottschalck, 1924; Kliewe, 1927). Microaerophilic diphtheroids were described by Eberson (1918) and Thomson and Thomson (1926).

10. *Relation to Hydrogen Ion Concentration.*—According to Dernby and Näslund (1922), Ishimori (1924), Kondo (1925) and Kondo and Nodaki (1925), various strains of *Myc. tuberculosis* and *leprae* (?) as well as saprophytic mycobacteria are very inconsistent in their reaction requirements, the limit of acidity even varying from pH 4·5 to pH 6·6; the limits of optimal reaction vary almost equally as much. *Cor. diphtheriae* will under otherwise optimal conditions tolerate an acidity of pH 5·2–5·3, with optimum at approximately neutral reaction (Dernby and Siewe, 1922; Walbum, 1922). Other corynebacteria do not seem to have been studied in this respect.

11. *Alleged Complex Life Cycles and Stabilized Variants.*—Metschnikoff (1888) first suggested that the tubercle bacillus might really be a developmental form of a higher organized fungus. Several authors of recent time (Vaudremer, 1921; Arloing and Dufourt, 1925; Kedrowsky, 1928; Karwacki, 1930) claim to have stabilized actinomyces-like variants of it. The same has often been alleged in *Myc. leprae*, but there seems to be no uniformity among the many organisms isolated from leprous lesions, which probably comprise corynebacteria, real mycobacteria, and species of *Proactinomyces*. Non-acid-fast variants, besides those mentioned in section 2 above (p. 20), have been described by Sweany (1926). Thompson and O'Brien (1920) claimed to have transformed *Myc. tuberculosis* and other acid-fast bacteria into microaerophilic diphtheroids by cultivation in mixture with *Bact. proteus*. Kuhn and Sternberg (1931) mention the stabilization of a coccoid, non-acid-fast "C-form" of the tubercle bacillus, said to have its counterpart among all other bacteria.* Kahn (1928) described a complex life cycle in *Myc. tuberculosis*, but this may, according to Ørskov (1932), largely depend on observational errors. The whole question seems intimately bound up with the problem of the existence of an ultra-microscopic, filtrable form of *Myc. tuberculosis*—a question where no decision has been arrived at in spite of the existence of an already enormous literature. *Actinomyces*-like variants of *Cor. diphtheriae* have been described by Cache (1901), Spirig (1904), and Concetti

* Recent critical studies by W. Kruse and co-workers have shown that the existence of Kuhn's "C-forms" must in general be regarded with much scepticism.

(cit. after Lehmann and Neumann, 1920). Variants of other character were described by Maver (1931). de Negri (1916) and Bergstrand (1918–19) described the wide range of pleomorphism in corynebacteria, without drawing any definite conclusions as to any cyclical development. Complex life-cycles, including the phenomena described by Löhnis (1922) as reproductive bodies, gonidia, and symplasm, were alleged to occur in corynebacteria by Almquist and Koraen (1918), Mellon (1920–26), and Grasset and Grasset (1930); the last-named authors, like Mellon (1926), describe also certain mycelial forms.

The Distribution of Mycobacteria and Corynebacteria in Nature.

Severin (1895) first observed acid-fast bacteria as saprophytes in manure. Soon afterwards, saprophytic mycobacteria were isolated from butter by Rabinowitsch (1897), Korn (1899), and Tobler (1901), and from dung and plant materials by Moeller (1898–99). More recently they have been shown to be common in milk (Albiston, 1930) and tap-water (Eichbaum, 1932). Their frequent occurrence in soil was first discovered by Herr (1901), later confirmed by Kersten (1909), Büttner (1926), Haag (1927), who also found them very common in other habitats, and Frey and Hagan (1932). The soil "mycobacteria" of several other authors were probably largely actinomycetes of the *Proactinomyces*-type (Haag, 1927; Jensen, 1932).

While the common occurrence of mycobacteria as saprophytes has been known for a long time, a quite different view has prevailed in regard to the corynebacteria, which have generally been considered more or less strict parasites and, perhaps for that very reason, hardly studied at all from other than medical points of view. McClure (1898) isolated a diphtheroid from milk, but this may have been one of the udder-corynebacteria of Steck (1932). The first isolation of a corynebacterium from other than animal habitats was reported by Honing (1912), but this organism was probably not a real corynebacterium (motile!). Harris and Wade (1915) found numerous corynebacteria in the air, and were the first to call attention to their probable frequent occurrence as saprophytes, a view which Eberson (1918) shared. Kisskalt (1917) isolated a corynebacterium from water, but did not later (Kisskalt and Berend, 1918) find such organisms as common saprophytes. Several of those organisms which Kisskalt and Berend recognized as corynebacteria are found in air, water, dung, milk, etc. (Lehmann and Neumann, 1927). Barratt (1924) isolated a serum-liquefying diphtheroid from an oyster. Klieneberger (1932) found corynebacterium-like organisms occurring as contaminants on agar plates. The present writer found corynebacteria occurring in quite surprising numbers in soil (Jensen, 1933). During the attempts to identify these and a number of mycobacteria isolated at the same time, I became aware that a large number of previously recorded bacteria, many of them not adequately described, probably belonged to the same group, which therefore seems to need a thorough revision from a general microbiological point of view. The present paper represents an attempt thereat.

Methods.

The soils from which isolations were made, as well as the medium for isolation, were described previously (Jensen, 1933). Some organisms, especially mycobacteria, were isolated by accumulation in a mineral nutrient solution with paraffin wax, and plating on some suitable agar medium. The following media were used for the morphological and cultural studies:

1. *Asparagine-agar*: dextrose 10·0 gm.; asparagine 1·0 gm.; K_2HPO_4 1·0 gm.; $MgSO_4$ 0·5 gm.; NaCl 0·5 gm.; agar 20·0 gm.; H_2O 1,000 c.c.; pH 7·0–7·2.—2.

Glycerine-agar: like (1), dextrose and asparagine replaced by glycerine, 40·0 gm., and ammonium lactate 5·0 gm.—3. *Nutrient agar*: meat-extract 5·0 gm.; peptone-Witte 10·0 gm.; NaCl 5·0 gm.; agar 20·0 gm.; H_2O 1,000 c.c.; pH 7·0–7·2.—4. *Dextrose-agar*: like (3), plus 1% dextrose.—5. *Nutrient gelatine*: like (3), agar replaced by 16% gelatine.—6. Broth: like (3), without agar.—7. *Sabouraud's agar* (de Negri, 1916): milk is boiled for 5 minutes with 0·2% HCl; to the neutralized filtrate are added 1% dextrose, 1% peptone, and 2% agar; pH 7·0–7·2; the addition of urea was found unnecessary, and was omitted.—8. *Milk-agar*: 4–5 c.c. of sterile milk and 8–10 c.c. of sterile melted 2·5% tap-water agar were mixed in a sterile Petri dish.—9. *Milk*.—10. *Potato*.—*Utilization of various nitrogen compounds* was tested in a solution containing 1% dextrose, the same mineral nutrients as media 1–2, and 0·2% of the following compounds: sodium nitrate, ammonium phosphate, asparagine, and peptone. It may here be mentioned that none of the organisms studied gave any positive evidence of fixation of free nitrogen; several strains grew feebly in the N-free solution, such as many bacteria may do at the expense of small impurities in the medium or volatile N-compounds of the atmosphere (Kondo, 1925; Braun and Goldschmidt, 1927). *Reduction of nitrate to nitrite* was tested in the above solution with nitrate at the end of the experiment, and in broth with 0·2% sodium nitrate, with Gries's reagent. *Inversion of saccharose* was tested in a similar solution with 2% saccharose and 0·2% sodium nitrate (or peptone for organisms not utilizing nitrate), by means of Fehling's reagent. *Diastatic action* was tested, by means of iodine solution, in plate culture on agar containing 1% soluble starch, 0·2% peptone, and the mineral nutrients of media 1–2. *Proteolytic action* was tested by formaldehyde titration of 4-weeks-old milk cultures. *Indol formation* was tested by the Ehrlich-Böhme method in a 2% peptone solution. *Haemolysis* was tested on blood-agar plates. *Relation to hydrogen-ion concentration* was tested in a solution containing: dextrose 10·0 gm.; peptone 2·0 gm.; KH_2PO_4 and K_2HPO_4 in varying proportions, 5·0 gm.; $MgSO_4$ 0·5 gm.; NaCl 0·5 gm.; H_2O 1,000 c.c.; pH adjusted, by means of HCl, to pH-values from 3·8 to 7·2. *Fermentative properties* were tested in the following medium, which is composed on the basis of the studies of Schroeder (1931), and which was found to give a much clearer picture of the acid- or alkali-formation than broth media: carbon compound 10·0 gm.; casein dissolved in 1n NaOH, 1 gm.; K_2HPO_4 0·2 gm.; $MgSO_4$ 0·5 gm.; NaCl 0·5 gm.; agar 20·0 gm.; H_2O 1,000 c.c. Bromo-thymol-blue in alkaline aqueous solution was added to a concentration of 0·005%, and the reaction was adjusted to pH 6·6–6·8. Slope-cultures were incubated for 20 days. The following compounds were tested: arabinose, dextrose, levulose, galactose, maltose, saccharose, lactose, glycerine, mannite, and dulcite. *Dissociation*: Two special media were used in attempts to enforce dissociation of the organisms: (*a*) ("lithium-solution") containing dextrose 1%, peptone, 0·5%, LiCl 1%, mineral nutrients as in media 1–2; and (*b*) ("uranium solution") containing dextrose 1%, asparagine 0·1%, uranyl nitrate 0·2%, same mineral nutrients. All media were sterilized at 110°C.; a period of 10 minutes was sufficient to ensure sterility, and caused no change in the reaction of the sugar media. Neither did the gelatine fail to solidify after this treatment. Gelatine cultures were incubated at 18–22°C., all others at 28–30°C., unless otherwise stated.

The familiar method of plate-streaking was used regularly for the isolation of variants. This technique has recently been very severely criticized by Klieneberger (1932), whose paper was not seen by me until most of the experimental

work had been finished. In order to get an idea of the dangers to be expected, 50 control plates (dextrose agar, Sabouraud's agar, milk-agar) were poured and streaked with sterile needle. Result after incubation at 28–30°C. for 4–5 days:

Plates remaining sterile	33	(66%)
Plates infected by mould	4	(8%)
„ „ „ yeast	2	(4%)
„ „ „ actinomyces	1	(2%)
„ „ „ bacteria	10	(20%)

Mostly only one bacterial colony appeared, rarely two, but one plate showed that type of contamination which Klieneberger mentions as particularly dangerous: multiple colonies—11 in this case—probably due to the rubbing out of infecting bacterial clumps. Although thus Klieneberger's warning is undoubtedly very timely, I am convinced that the variants dealt with here are not contaminants, since each of them was recognizable as a modification of the strain from which it had been derived, and was obtained from this particular strain only. Variants which one would be inclined to regard as alien to the nature of mycobacteria and corynebacteria, such as spore-formers, motile forms or true cocci, were never found.

Authentic cultures of the following organisms were used for comparison: *Myc. tuberculosis*, avian type, from the Department of Medical Bacteriology, Sydney University; *Myc. phlei*, from the culture collection of the McMaster Laboratory of Animal Health, Sydney University; *Myc. coeliacum*, from the Rothamsted Experimental Station, Harpenden, England; *Cor. pseudodiphtheriticum*, one strain from the McMaster Laboratory, another from the Department of Medical Bacteriology; *Cor. equi*, two strains from the McMaster Laboratory, one isolated by Dr. Bull, Adelaide, another isolated at the Glenfield Veterinary Research Station, N.S.W.; *Mierobacterium flavum*, *lacticum* and *liquefaciens*, from the Biotechnical-Chemical Laboratory, Polytechnical College, Copenhagen, Denmark; *Aplanobacter michiganense*, from the Faculty of Agriculture, Dept. of Plant Pathology, Sydney University; *Cellulomonas fimi*, from the New Jersey Agricultural Experiment Station, U.S.A.; *Bact. fulvum*, from the National Collection of Type Cultures, Lister Institute of Preventive Medicine, London. I wish to express my sincere thanks to the scientists at the various institutions who have placed these cultures at my disposal.

The Genus Mycobacterium.

The strains of *Mycobacterium* formed two morphologically and biologically fairly distinct subgenera.

Subgenus *A*.—This group, comparatively rare in soil, resembles *Myc. tuberculosis* morphologically and conforms with the saprophytic mycobacteria of Haag (1927). These organisms are morphologically characterized by a typical combination of "snapping" and "slipping" cell division, the latter type superseding the former. During the first stages of growth after transfer to fresh medium the young cells occupy angular positions, but soon the ends of the cells separate at the place of division, the cells bend and continue to grow past each other, thereby, especially in *Myc. tuberculosis*, producing characteristic wisp-like colonies composed of bundles of parallel, uneven-sided rods. Text-figures 1–3 show good examples of this mode of growth. True branching occurs sometimes in the early stages, but never frequently. The acid-fastness against 20% sulphuric acid is well developed in the saprophytic species, although not so strongly as in *Myc. tuberculosis*. The cells are generally longer in young than in older cultures,

but this is not always equally pronounced. I have never observed any filaments or branching forms in old cultures. There is also little tendency to formation of swollen, club-like cells, although an irregular thickness is common. Acid-fast organisms with a marked club-formation have been described by Korn (1899) and Tiedemann (1930), but particularly the latter organism seems to be a *Proactinomyces*, in which such cell types are common (Ørskov, 1923; Jensen, 1932); indeed, the organism which Tiedemann considers identical with *Myc. luteum* Söhngen is very suggestive of the yellow variant of *Proact. polychromogenes*. In physiological respect these bacteria are, like *Myc. tuberculosis*, able to grow well on simple N-compounds, but they show no preference for glycerine as a source of energy. They utilize paraffine readily, but do not invert saccharose, hydrolyze starch or decompose cellulose. They are strictly aerobic, and show no or only a very faint acid-formation in sugar-media. They are immotile and gram-positive, and they show no haemolytic or, as discussed in detail below, proteolytic effect.

MYCOBACTERIUM LACTICOLA Lehmann and Neumann.

Synonyms: *Myc. berolinensis, friburgensis,* and *graminis* Bergey (1923–30).*— Other synonyms, see Haag (1927).

One strain, isolated from heavily manured garden soil.

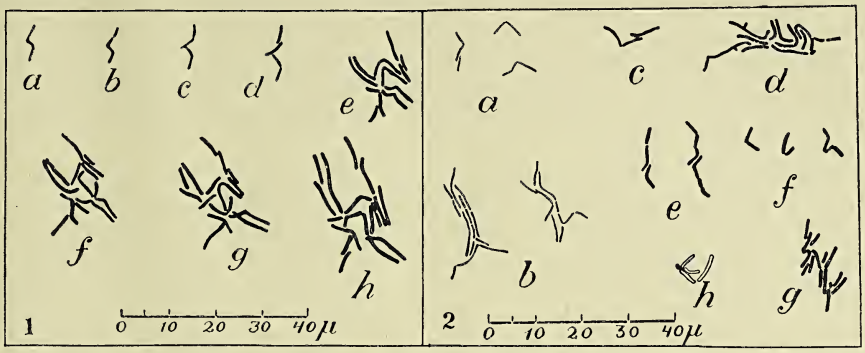
Morphology.—This organism shows by direct agar-microscopy, according to Ørskov (1923), a beautiful combination of snapping and slipping growth on asparagine-agar as well as in protein-media (Text-fig. 1). 2–3-day-old colonies show short projections of parallel cells, and on nutrient agar one sees at this time a number of small refractive external granules on the cells, visible by high focussing and doubtless identical with the rudimentary aerial mycelium in certain species of *Proactinomyces* (Jensen, 1932). 18–24-hours-old cells are long and slender, 0·5–0·7μ × 2–8μ, not staining well with ordinary dyes, after 2–3 days shorter, 1·2–3·0μ, and after 6–7 days almost coccoid. The organism is strongly acid-fast in synthetic as well as protein media, but only faintly so in dextrose-asparagine-solution of pH 5·0. The morphology does not vary much according to medium or temperature. Branching, if present at all, is quite infrequent.

Cultural characters.—Asparagine-agar: good growth, edges entire, surface convex, glistening, at first smooth, after 10–14 days somewhat folded; growth opaque, at first white with creamy tinge, later becoming ochre-yellow; consistence at first sticky, gum-like, becoming soft and mealy. *Dextrose-agar*: very much like previous, more rapid growth, consistence pasty, colour less intense. *Potato*: fair growth, restricted, convex, smooth, becoming slightly folded, glistening, light ochre-yellow. *Gelatine*: thin filiform growth in stab; small, round, convex, smooth, glistening, greyish-yellow surface colony; no liquefaction. *Broth*: faint uniform turbidity, small soft white sediment which gradually becomes yellow; thin, dry, silky surface pellicle after 3–4 weeks. *Milk*: small white granules on the surface and along the tube, later a dry, pale yellow surface pellicle; the milk is not coagulated, but after 3 weeks slightly cleared, after 10 weeks almost transparent, viscid, alkaline; at 37°C. the clearing is very strong after 3–4 weeks.

Physiological characters.—Nitrate, ammonia, asparagine and peptone are utilized almost equally well. Reduction of nitrate, doubtful. Slight production of acid from levulose. Optimal reaction, pH 6·8–7·2. Dextrose-NH_4Cl-solution is

* It is not obvious why Bergey has disregarded the priority of the unquestionably valid species-name *lacticola*.

acidified to pH 3·7–3·8 in 3 weeks. Excellent growth at 37°C. Growth stops at pH 4·3–4·6.



Text-figure 1.—*Myc. lacticola.* Successive stages in development on asparagine-agar, room temp. Direct agar-microscopy. *a-d* 20-24 hours, 2-hour intervals; *e-g* 44-48 hours, 2-hour intervals.

Text-figure 2.—*a. Myc. tuberculosis* (avian), Sabouraud's agar, 2 days, 37°C.; *b.* Same, 4 days, 37°C.; *c. Myc. phlei,* authentic, asparagine-agar, 1 day, 28°C.; *d.* Same, 3 days, 28°C.; *e. Myc. phlei* 282, plane type, glycerine-agar, 2 days, 20°C.; *f.* Same, perrugose type, asparagine-agar, 1 day, 20°C.; *g.* Same, 2 days, 20°C.; *h.* Same, with aerial "mycelium" (heavily shaded).—All by direct agar-microscopy.

Dissociation.—The above description agrees with the "plane" type of *Myc. lacticola* (Lehmann and Neumann, 1911–27; Haag, 1927). A variant of a somewhat "perrugose" character was obtained by plating from an 80-days-old culture in dextrose-peptone-solution of pH 4·6; its young agar cultures differed from the original by showing a more flat and spreading growth with lobate edges and a dull, finely rugose surface. A definitely "perrugose" variant was obtained by plating from 7-months-old culture in lithium-solution and from 2-months-old culture in uranium-solution. This variant produces in agar-media a spreading, flat, strongly wrinkled, dry and dull growth of a tough consistence; broth and other liquid media remain clear with a dry, thick pellicle and flaky sediment. Morphologically these variants are like the original type.

MYCOBACTERIUM PHLEI Lehmann and Neumann.

Synonym: *Myc. stercusis* Bergey (1930). Other synonyms, see Haag (1927).

One authentic strain; one (282) isolated from soil from Griffith, N.S.W.

Morphology.—Both strains appear as small, fairly straight rods, 0·5–0·7μ × 1·5–4·0μ, not varying much in different media or at different ages. The authentic strain shows distinct snapping and slipping growth, whereas 282 has a tendency to produce chain-like figures, somewhat like the "Harnbacillus" of Miehe (1909). Branching, although present, is rare (Text-fig. 2). The authentic strain is more acid-fast than 282, which after 1 day on asparagine- or dextrose-agar has only a minority of acid-fast cells; after 2–3 days the acid-fastness is good, but markedly granular and belt-like, in milk after 7 days complete, but in broth only partial.

Cultural characters.—The authentic strain, which is markedly perrugose, bears little resemblance to the plane soil strain. *Asparagine-agar*: good growth, becoming abundant; authentic strain spreading, edges lobate, surface flat, dull, dry, rugose, 282 restricted, convex, smooth, moist, glistening; both white, becoming ochre-yellow. *Dextrose-agar*: like previous, still better growth, authentic strain

more strongly wrinkled. *Potato*: good growth, becoming abundant, greyish-yellow; authentic granular, 282 smooth and glistening. *Gelatine*: granular yellowish growth in stab; wrinkled yellow surface colony; no liquefaction. *Broth*: authentic strain leaves the broth clear with dry white, later yellow pellicle; 282: uniform turbidity, later slimy cream-coloured pellicle and sediment. *Milk*: fragile yellowish pellicle on surface and along the tube; milk slowly cleared, semi-transparent and viscid after 45–60 days; reaction alkaline. 282 grows in all media more rapidly than the authentic strain.

Physiological characters.—Both strains utilize nitrate and ammonia well, although asparagine and peptone are superior. Nitrate is reduced to nitrite, strongly by the authentic strain, feebly by 282. The authentic strain produces no acid from sugars; 282 shows a faint acid-production in glycerine and mannite, and gives in old cultures a doubtful reaction in arabinose and dextrose. Optimal reaction, pH 6·8–7·2; 282 continues to grow at pH 4·3, and acidifies dextrose-NH_4Cl-solution to pH 3·8–3·9; the corresponding figures for the authentic strain are pH 5·3–5·6 and pH 4·2. Excellent growth at 37°C.

Dissociation.—A perrugose variant of 282 was obtained in great abundance by plating from 45-days-old culture in lithium-solution and from 80-days-old cultures in dextrose-peptone-solution of pH 4·3–4·6; only the original plane type was recovered from solutions of pH 6·8–7·2. This variant was culturally very much like the authentic strain, from which it differed only in a more rapid growth, a less strong acid-fastness, and some biochemical properties as stated above; like this it showed a distinct slipping growth (Text-fig. 2).

Subgenus *B.*—This group occurs more commonly in soil than the previous, from which it differs in its mode of cell division: the snapping growth is here predominant, and the slipping growth is little in evidence, sometimes not noticeable at all. Most organisms show a characteristic cytomorphosis: cells of the first generations after transfer to fresh medium ("embryonic forms", Henrici, 1928) are long, uneven-sided, sometimes branching rods which during the following stages of cell division gradually grow shorter ("mature forms"), until they finally appear as coccoid forms, often united in small clumps or short chains ("senescent forms"). *Myc. coeliacum* (Gray and Thornton, 1928; Jensen, 1931) may be regarded as the type-species of this group. It is worth noticing that this transformation from branched rods to cocci had been described previously by Ward (1898), who thought he was dealing with a minute fungus, and whose contribution seems to have been overlooked by subsequent workers. The acid-fastness is much weaker than in subgenus A, often noticeable only in milk-cultures, and it is not increased in subcultures from paraffine-cultures, as reported by Haag (1927). Cells of varying thickness and slender club-like forms are common, but typical cystites are rarely produced. Branches arise in precisely the same manner as in *Proactinomyces* (Jensen, 1932), and granules of aerial "mycelium" are common. Some strains utilize non-protein-nitrogen badly, and fail to utilize paraffine. Acid-production from sugars, although mostly weak, is stronger than in subgenus A.

Mycobacterium coeliacum Gray and Thornton.

Synonym: *Flavobacterium coeliacum* (Gr. and Th.) Bergey (1930).

One authentic strain, and three isolated from soil: AI from lucerne soil, AIII from garden soil, 18 from grass soil.

Morphology.—All strains show after 18–24 hours on asparagine or nutrient agar a predominant snapping growth, on glycerine-agar also a certain amount of

slipping (Pl. i, figs. 3–4). The length varies from 2μ to 10μ, occasionally longer; the cells are shorter in protein media than in synthetic media (Pl. i, figs. 1–2). After 2 days the cells are shorter, 0·8–2·0μ, many quite coccoid (Pl. i, fig. 6). In older cultures only cocci are seen. Branching occurs sometimes in the early stages (cf. Jensen, 1931), particularly on glycerine-agar (Pl. i, fig. 3). Granules of aerial "mycelium" are common. No strain shows more than a trace of acid-fastness in synthetic or nutrient agars, but all exhibit a partial acid-fastness in milk after 3–10 days, particularly when decolorized for 15–30 seconds with 5% sulphuric acid. The strains agree well in morphological respect, except that strain 18 is somewhat thinner, 0·6–0·8μ against 0·7–1·0μ, and the authentic strain forms long filaments with cystite-like swellings in egg-media (Pl. i, fig. 7).

Cultural characters.—These differ more than the morphological ones. The original soil strains of the plane type did not resemble the authentic much, but the perrugose variants of two of them differ from this only in a less ready assimilation of non-protein-nitrogen, and a few minor points. The peculiar lobate growth in gelatine stabs, mentioned by Gray and Thornton (1928), was seen neither in the authentic nor in the soil strains. Apparently this character is easily lost, and moreover it must be remembered that Gray and Thornton based their description on one strain only. *Asparagine-agar*: authentic strain excellent growth, spreading, flat, opaque, edges undulate, surface folded, faintly glistening, cream-coloured, becoming greyish-yellow; soil strains only scant to fair growth (least in AIII), narrow, convex, smooth, glistening, semi-transparent, white becoming cream-coloured. *Dextrose-agar*: abundant growth; authentic strain like previous medium, colour more pinkish-orange, consistence crumbly; soil strains restricted, convex, edges even, surface smooth, glistening, at first moist and semi-transparent, white to cream-coloured, later dry and opaque with pinkish-orange tinge; consistence pasty. *Potato*: abundant growth, spreading, somewhat folded, dirty cream-coloured, later with orange tinge; authentic strain dry, dull and crumbly, soil strains moist, glistening and pasty. *Gelatine*: thin cream-coloured growth in stab, first granular, in old cultures finely arborescent; pinkish-cream-coloured spreading surface colony, flat, sometimes convex, while young, with lobate edges and radially wrinkled surface. No liquefaction. *Broth*: authentic strain produces at first a faint turbidity, with broken cream-coloured pellicle and granular sediment, later clearing; soil strains similar, with stronger turbidity. *Milk*: pinkish-cream-coloured flakes along the tube, later sediment and fragile pellicle of the same colour. Milk is slightly cleared after 3–4 weeks, semi-transparent and thickened after 45–60 days, reaction alkaline.

Physiological characters.—The strains differ mainly in their relation to non-protein-N, which is utilized well by the authentic strain, less readily by the soil strains, especially AIII. The authentic strain and AI reduce nitrate strongly to nitrite, the others feebly or not at all. The authentic strain utilizes paraffine readily with nitrate as a source of N, AI and AIII less readily unless provided with peptone, 18 apparently not at all. All strains produce acid from glycerine and mannite, 18 and AI also from levulose, AIII from saccharose. Optimal reaction, pH 6·2–7·2. Growth stops at pH 4·3–4·6. Dextrose-$\bar{N}H_4Cl$-solution is acidified to pH 3·5–3·7, by AIII only to pH 4·5–4·6. No growth, or only very scant, at 37°C.

Dissociation.—Strains AI and AIII gave perrugose variants, the former from the surface pellicle of an 18-days-old broth culture and from 205-days-old culture in lithium-solution, the latter from an 82-days-old culture in dextrose-peptone-

solution of pH 4·9; from solutions of pH 6·8–7·2 and from lithium-solution only the original plane type of this strain was recovered. The perrugose variants were morphologically like the original plane. Culturally they appeared, except for a more feeble growth on asparagine-agar, much like the authentic strain, producing on dextrose-agar a still more wrinkled, dry and dull, cream-coloured to greyish-yellow, crumbly growth, and leaving broth clear with thick fragile pellicle and flaky sediment. The authentic strain produced a plane variant after 130 days' cultivation in uranium-solution; this variant produced in agar media a smooth, glistening pinkish-orange growth, resembling the soil strains. This is the only instance I have observed of the perrugose type changing into the plane.

A number of previously described organisms, the true nature of which has not been realized, are probably closely related to or perhaps identical with *Myc. coeliacum*. These are: (1) the "false Bacterium" of Ward (1898); (2) *Bac. Berestnewii* Lepeschkin (1903). These two organisms seem to be longer and more branched in their young stages, and may come closer to *Proactinomyces*. (3) Bacillus No. 2 Bertani (1913); (4) *Actinococcus cyaneus* Beijerinck (1916); (5) *Nitrobacter opacus* Sack (1924; instructive microphotograph!); (6) nitrifying organism of Runow (1929).

MYCOBACTERIUM RUBROPERTINCTUM (Hefferan) Ford.

Synonyms: *Bacillus rubropertinctus* Hefferan (1904); *Serratia rubropertincta* (Grassberger) Bergey (1923–30).*

Two strains, from red soil from Griffith (279) and grass soil from Sydney (M).

Morphology.—This species shows an almost exclusive snapping growth on agar (Text-fig. 3). 18–24-hours-old cells are rod-shaped, 0·5–0·8μ × 1·5–5·0μ, in beautiful angular arrangement (Pl. i, fig. 8), after 2–3 days nearly coccoid, 0·6–0·8μ. The cocci are produced more rapidly in protein agar than in synthetic agar. Young colonies are often star- or burr-shaped, with projections of cells which remain rod-shaped longer than those in the interior. Branching does not occur in most media, but granules of aerial mycelium are sometimes seen (Text-fig. 3). On glycerine-agar there is, after 2–3 days, a tendency to formation of longer, club-shaped, sometimes branching cells around the edges of the colonies. No acid-fastness in agar-media after 1 day, sometimes a trace after 3–7 days. Cultures in milk are partially acid-fast after 3–7 days.

Cultural characters.—Asparagine-agar: good growth, restricted, convex, edges entire, surface smooth and glistening; growth opaque, pasty, first light coral-red, becoming very intense red. *Dextrose-agar*: abundant growth, narrow, convex, edges entire, surface smooth, glistening, opaque, orange-red, gradually becoming very intense scarlet. Strain M forms many small round white to pink secondary colonies after 3–4 weeks; 279 did the same immediately after its isolation. In sugar-free nutrient agar fair growth only, less intense red, with fewer secondary colonies. *Potato*: abundant growth, spreading, flat, somewhat granular, faintly glistening, first coral-red, then, after 6–8 days, dull orange, rather pale after 3 weeks. *Gelatine*: growth in stab at first thin, after 1–2 months quite heavy, granular to finely arborescent, yellow; small, convex, lobate, folded red surface colony; no liquefaction. *Broth*: faint uniform turbidity, later clear; small sediment and surface scales, first pink, becoming dull red. *Milk*: thick, fragile, dull coral-red pellicle and sediment; milk definitely cleared and somewhat viscid after 3–4

* The name *rubropertinctus* is due to Hefferan (1904), and not to Grassberger, who did not name the organism.

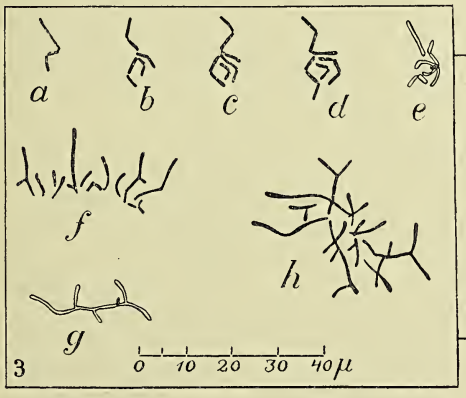
weeks; reaction alkaline. At 37°C. the milk is almost transparent after 4 weeks.

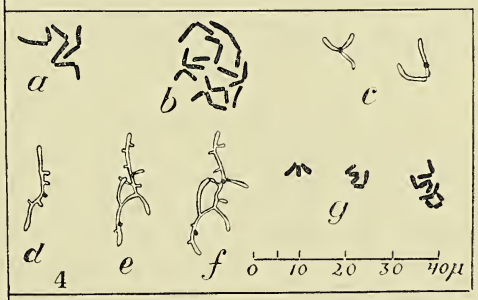
Physiological characters.—Nitrate is utilized readily, and ammonia and asparagine are almost as good sources of N as peptone. Nitrate is not reduced to nitrite. Paraffine is utilized very readily by 279, hardly at all by M. Acid is produced from saccharose and glycerine; 279 gives a doubtful reaction in dextrose and mannite. Optimal reaction, pH 6·8–7·2. Growth stops near pH 4·9. Dextrose-NH_4Cl-solution is acidified to pH 4·0–4·1 by 279, to pH 4·6–4·7 by M. Excellent growth at 37°C.

Dissociation.—Two separate phenomena of dissociation were observed.

i. *Perrugose variants.* Such a variant of 279 was obtained by plating from 2-months-old milk-culture and from 140-days-old culture in dextrose-peptone-solution of pH 6·8. In dextrose-agar cultures of M such a variant arose immediately after isolation as dull, flat outgrowths from the edge of the growth, and it was also obtained from 6-days-old culture in uranium-solution. These perrugose variants are morphologically like the plane, rather shorter in the rod-stage. In solid media they produce a flat, spreading, highly wrinkled, dry and dull, crumbly growth of a deep carmine-red colour, without secondary colonies, in broth a flaky cream-coloured sediment and coral-red scales on the surface, leaving the broth clear. They seem to correspond to the "dry" strains of Grassberger (1898).

ii. *Pink myceloid variants.* Plating from the white secondary colonies in single-cell cultures of M on dextrose-agar yielded a constantly pink variant of a plane type; a perrugose form of this was obtained by plating from a 10-weeks-old milk culture. A pink perrugose variant of 279 was obtained by plating from a 30-days-old single-cell culture in uranium-solution. These pink variants, plane as well as perrugose, differ morphologically from the red ancestral organisms. Their cells in asparagine- and dextrose-agar are longer, more frequently branching (Text-fig. 3), and produce cocci less rapidly. The most striking difference is





Text-figure 3.—*Myc. rubropertinctum.* Direct agar-microscopy. *a-d.* Successive stages in development, dextrose-agar, 22°C., 19-24 hours; *e.* Same, gelatine, 1 day, 20°C.; aerial "mycelium" heavily shaded; *f.* pink variant, dextrose-agar, edge of colony, 2 days, 25°C.; *g.* Same, glycerine-agar, 1 day, 25°C.; aerial "mycelium" heavily shaded; *h.* Same, 2 days, 25° C.

Text-figure 4.—*a-f. Myc. equi. a-b.* Successive stages of development, dextrose-agar, 25°C., 17 and 22 hours; *c.* Same, dextrose-agar, 26 hours, 20°C.; aerial "mycelium" heavily shaded; *d-f.* Perrugose type, dextrose-agar, 23°C.; 17-24 hours. *g. Myc. flavum,* casein-agar, 18 hours, 18°C.—All direct agar-microscopy.

seen in glycerine-agar, where after 1–3 days they produce actual small mycelia with numerous granules of aerial "mycelium" (Text-fig. 3), exactly as in certain species of *Proactinomyces* (Jensen, 1932). The mycelia divide into rods, and then cocci after 4–5 days. The M-variant is flesh-pink to pale coral-red in solid media, the 279-variant rose-pink in asparagine-agar, deep orange in dextrose-agar.

The red plane and perrugose types agree well with Hefferan's (1904) fairly good description of *Bac. rubropertinctum*, an organism isolated, but not named, by Grassberger (1898), and later, on account of its partial acid-fastness, transferred to the mycobacteria by Ford (1927). *Bact. rubrum* Migula (1900) may be identical, but is not sufficiently well defined to be identified with the present organism. Haag (1927) mentions a *Bact. rubrum* as capable of decomposing paraffine and otherwise appearing as a mycobacterium. *Myc. eos* Büttner (1926), although not very completely described (Haag, 1927), appears somewhat similar, but according to the statements of Haag, who recognized it as a variant of *Myc. lacticola*, it seems to exhibit the slipping growth of subgenus A. *Myc. rubrum* Söhngen (1913) is mentioned by Lehmann and Neumann (1927) as possibly identical with the weakly acid-fast *Myc. eos*, but according to Gray and Thornton (1928) *Myc. rubrum* is definitely acid-fast and thus hardly identical with the present organism either; maybe it is merely a strongly pigmented variety of *Myc. phlei.*

In connection with this species we may mention two strains which are closely related, but yet seem to be too aberrant to be included in the species-group.

Mycobacterium 272.

Isolated from red soil, Griffith. Morphologically and tinctorially it resembles *Myc. rubropertinctum*, still less acid-fast, and rather shorter and less branched in glycerine-agar. Its appearance in various media is also similar, but entirely devoid of red pigment, greyish-yellow to light ochre-yellow, like *Myc. lacticola*, from which it is otherwise quite different. It forms a perrugose variant like *Myc. rubropertinctum*, which it also resembles in physiological respect, differing from it in reducing nitrate to nitrite and in forming acid from dextrose and galactose. Paraffine is utilized fairly well. The organism is probably to be regarded as a non-pigmented variety of *Myc. rubropertinctum.*

Mycobacterium Bb.

From light sand soil under grass, Sydney. It is culturally and tinctorially somewhat similar to *Myc. rubropertinctum*, rather less acid-fast, and produces a less vigorous growth of a less intense red colour. Morphologically it differs in lacking the typical cytomorphosis with transformation from rods to cocci. It appears in nutrient agar at most ages as short rods with pointed ends, 0·5–0·6$\mu \times$ 3–4μ, with strong belt-staining and no branches. It reduces nitrate to nitrite, produces acid from dextrose and saccharose, and utilizes paraffine only with peptone as a source of N; no growth takes place at 37°C. A morphologically similar perrugose variant was obtained by plating from a 118-days-old culture in dextrose-peptone-solution of pH 5·3; it produces a rather pale coral-red to orange growth. This strain is somewhat suggestive of *Bact. latericeum* (Adametz), which is mentioned by Lehmann and Neumann (1927) as possibly a corynebacterium (cf. Kisskalt and Berend, 1918), but which is not sufficiently well defined to be identified with the present; moreover, the identity of *Bact. latericeum* seems dubious (Hefferan, 1904). We shall therefore regard this strain as a variety of *Myc. rubropertinctum.* The same may possibly be true of the badly defined *Bact. roseum* and *Bact. Winkleri* (Migula, 1900).

MYCOBACTERIUM EQUI (Magnusson), n. comb.

Synonyms: *Corynebacterium equi* Magnusson (1923); *Cor. (pyogenes) equi roseum* Lütje (1923). The *Cor. pyogenes (equi)* Miessner and Wetzel (1923), which Lehmann and Neumann (1927) identify with Magnusson's organism, is probably different, and seems to be a variety of the familiar *Cor. pyogenes* (cf. Brown and Orcutt, 1920).

Five strains were studied: one authentic, originally isolated by Dr. Bull (1924), two (N and A) isolated from garden soils, one (M) from grass soil, and one (125) from alluvial clay from Bathurst. The soil strains and the authentic strain are similar in every character studied, and agree well with the original descriptions by Magnusson and Bull. A *Bact. aurantium-roseum* Honing (1912) seems to bear a close resemblance to the present. Apparently the organism is a widespread soil saprophyte which under certain conditions acquires pathogenic properties. Its acid-fastness and particularly its very close relationship to *Myc. coeliacum* show that it has its place in the genus *Mycobacterium* rather than in *Corynebacterium*.

Morphology.—All strains produce in nutrient-agar after 16–24 hours at 20° to 30°C. long rods, mostly 0·8–1·0μ × 2–8μ, multiplying by typical snapping growth without any slipping; longer, branched rods with granules of aerial "mycelium" are occasionally seen (Text-fig. 4). After 2 days and in older cultures one sees only short, oval- to pear-shaped rods and cocci, 0·8–1·2μ × 1·0–1·5μ (Pl. i, fig. 9), often in short chains. A number of rods, some of them branching, may be found in broth and milk after 2–3 days. At 28–30°C. the rods in dextrose-agar are after 24 hours considerably shorter than at 20–22°C.; at 37°C. the cocci predominate already after one day (cf. Thomson and Thomson, 1926). The present is the most acid-fast species of subgenus B. In dextrose-agar and egg-medium numerous cells are partially acid-fast after 3–7 days, but not after one day. In milk there is after 3–7 days a quite strong acid-fastness, also towards 20% sulphuric acid: most cocci retain the stain completely, while the rods take the counterstain. The microscopical picture is very similar to that given by Martinaglia (1932).

Cultural characters.—*Asparagine-agar*: very scant growth; narrow, flat, thin, colourless streak, in old cultures sometimes becoming slightly yellowish and granular, in strain N with grey centre. *Dextrose-agar*: good and very characteristic growth, after 2–3 days narrow, convex, edges entire, surface smooth and glistening, very moist, semi-transparent, colourless or light cream-coloured with very pale pink tinge, after 5–6 days more dry and opaque, assuming a light pinkish-orange colour, and after 16–20 days quite firm and opaque, with finely rugose surface and finely myceloid edges. *Potato*: scant to fair growth, spreading, flat, thin, very moist, pink, gradually (7–14 days) growing more dry and opaque, granular, light dull orange. *Gelatine*: thin filiform to granular growth in stab, in old cultures becoming finely arborescent; small, round, convex, smooth, pinkish-cream-coloured surface colony; no liquefaction. *Broth*: faint uniform turbidity, small soft cream-coloured sediment. *Milk*: fine cream-coloured flakes along the tube, later cream-coloured to pink sediment. Milk is thickened, but only very slightly cleared, after 80–90 days; reaction faintly acid.

Physiological characters.—Non-protein sources of N are but very poorly utilized. Reduction of nitrate to nitrite is doubtful in strain M, faint in 125, strong in the others. Paraffine does not seem to be utilized even with peptone as a source of N, except perhaps in strain M. Only two strains (authentic and 125) show a faint production of acid from dextrose. Optimal reaction, pH 6·2–7·2.

Growth stops at pH 4·3–4·6; the authentic strain grows still at pH 4·3. The strains grow fairly well at 37°C., but apparently better at 28–30°C.

Dissociation.—Strains 125 and N yielded perrugose variants, the former from secondary colonies in old culture on dextrose-agar, the latter from a 112-days-old culture in dextrose-peptone-solution of pH 4·9. These variants produced on dextrose-agar a wrinkled, dull, warty growth of a rather hard, crumbly consistence, and in gelatine a flat, erose surface colony and a definitely arborescent growth in the stab. Morphologically they are distinguished by forming, after 1–2 days, long, curved, richly branching filaments which definitely approach a mycelium (Text-fig. 4) and carry numerous granules of aerial "mycelium". The young "mycelia" adhere firmly to the agar and show marked belt-staining; after 3–4 days they divide, like the plane type, into cocci which have a pronounced tendency to adhere together in chains.

In connection with this species we may mention the strain isolated at Glenfield Veterinary Research Station and identified as *Cor. equi*, which was studied for comparison. It resembles *Myc. equi* in a general way, but its rods in the young stage in dextrose-agar are considerably shorter ($0·8–1·0\mu \times 1·5–4·0\mu$), it is less acid-fast, grows hardly at all on potato (only a few small red granules after 3–4 weeks), and produces an intensely red growth on dextrose-agar, like *Myc. rubropertinctum*, which it also resembles in its growth in gelatine. Physiologically it resembles *Myc. equi*, but ceases to grow at pH 4·9–5·3, and shows a stronger acid-production in dextrose. It bears some resemblance to *Bact. erythromyxa* (Zopf) Migula (1900), but since this is not a well-defined species, the present strain cannot be identified with it, and must be left as an uncertain form which seems to occupy an intermediate position between *Myc. equi* and *Myc. rubropertinctum*.

MYCOBACTERIUM FLAVUM (Orla-Jensen), n. comb.?

Synonym: *Microbacterium flavum* Orla-Jensen (1919).

Morphology.—Cells in 18–24-hours-old cultures on dextrose-agar or Sabouraud's agar are rod-shaped or bluntly cuneate, $0·6–0·8\mu \times 1·5–3·5\mu$, multiplying by purely snapping growth (Text-fig. 5). Granules of aerial mycelium are sometimes visible. Contrary to the statements of Wittern (1933), I have always found this organism to exhibit a very typical angular arrangement in stained preparations, which is also demonstrated by Orla-Jensen's microphotographs (1919, Pl. 1). In older cultures there is no very typical formation of cocci, but the cells are always shorter and plumper, up to $1·0–1·2\mu$ thick, belt-staining or approaching a coccoid shape (Pl. i, fig. 10), in milk definitely club-shaped. This is the least acid-fast of all mycobacteria. In 2–3-days-old agar cultures some cells appear violet with red granules after staining with hot carbol-fuchsin, differentiation with 5% sulphuric acid, and counterstaining with aqueous methylene blue. In milk after 6–10 days many club-shaped cells appear deep violet, the rest as pure blue rods and cocci.

Cultural characters.—Asparagine-agar: trace of growth only; small isolated dewdrop-like colonies. *Dextrose-agar*: fair growth, narrow, convex, smooth, glistening, opaque, light ochre-yellow. *Sabouraud's agar*: similar appearance, more vigorous growth, crumbly consistence. *Potato*: fair growth, restricted, later spreading, slightly folded, glistening, opaque, bright ochre-yellow. *Gelatine*: thin granular growth in stab, gradually becoming quite thick, yellow; small raised and wrinkled, ochre-yellow surface colony; no liquefaction. *Broth*: faint uniform turbidity, clear after 4 weeks; small soft cream-coloured sediment, becoming viscid and yellow. *Milk*: small ochre-yellow sediment; milk remains unchanged.

Physiological characters.—Non-protein sources of N are hardly utilized at all. Nitrate is reduced to nitrite. Starch seems to be slightly hydrolyzed. Paraffine is not utilized. Acid is produced from dextrose, levulose, galactose, and glycerine; more detailed studies of the fermentative properties are due to Orla-Jensen (1919) and Wittern (1933). Optimal reaction, pH 5·6–6·8. Growth stops at pH 4·9–5·3. At 37°C. fair growth, but less than at 28–30°C.

The morphology of this bacterium shows conclusively that it belongs naturally with the genera *Mycobacterium* and *Corynebacterium*, and the fact that it produces lactic acid would hardly justify the placing of it in a special genus *Microbacterium* (Orla-Jensen, 1919). Actually the biochemistry of the acid-production by the real corynebacteria seems never to have been studied in detail, and they may, for all that we know, produce lactic acid also. Otherwise it is quite difficult to find a natural place for this organism. It is admittedly very different from the familiar mycobacteria of subgenus A, and appears less acid-fast than several true corynebacteria (Haag, 1927). The main reason why it has here—only tentatively—been included in *Mycobacterium* is that, in its general morphological and biological aspects, it seems to attach itself naturally to organisms like *Myc. coeliacum* and *Myc. equi*, and it seems to represent the extreme, corynebacterium-like end of a spectrum of mycobacteria with *Myc. tuberculosis* at the opposite end.

Common Properties of the Mycobacteria.

All mycobacteria of subgenus B, as well as A, are non-motile and grampositive. They do not invert saccharose, decompose cellulose, hydrolyze starch (with the possible exception of *Myc. flavum*), or produce indole, although they often give a positive Salkowski-reaction. Neither do they show any haemolytic or proteolytic effect. As mentioned in the introduction, most mycobacteria produce very little or no acid, and Merrill (1930) considers them incapable of any partial cleavage of sugars or other carbon compounds. This is doubtless true of the organisms studied by Merrill—all typical acid-fast representatives of subgenus A—but not of the organisms of subgenus B; also the results obtained here with *Myc. lacticola* and *Myc. phlei* seem to indicate that at least some strains of acid-fast bacteria will produce small amounts of acid (cf. Birch-Hirschfeld, 1932, and Thomson, 1932). There seems to exist a certain inverse correlation between acid-fastness and power of acid-production, as shown below:

Organism.	Acid-fastness.	Acid-production.
Myc. tuberculosis	Perfect.	None.
„ *lacticola*	Very good.	Faint in levulose.
„ *phlei*, authent.	Very good.	None.
„ „ 282	Good.	Faint in glycerine and mannite.
„ *equi*	Weak to fair.	Faint, or none, in dextrose.
„ Glenfield-Str.	Weak.	Strong in dextrose.
„ *coeliacum*	Weak.	Glycerine, mannite, some strains in levulose, saccharose.
„ *rubropertinctum* ...	Weak.	Saccharose and glycerine.
„ 272	Very weak.	Glycerine, dextrose, galactose.
„ Bʙ	Very weak.	Dextrose and saccharose.
„ *flavum*	Trace.	Dextrose, levulose, galactose and glycerine.

Most saprophytic mycobacteria of both subgenera show a characteristic behaviour in milk culture, where they produce a slow clearing without any real coagulation, but gradually rendering the milk semi-transparent, opalescent, viscid and in 2–3-months-old cultures even quite gelatinous.* This peculiar change, which

* This may be the "coagulation" referred to by Bertani (1913).

has been commented on by very few authors (e.g., Lehmann and Neumann, 1920, on *Myc. lacticola*), and which is also produced by the partially acid-fast proactinomycetes (Jensen, 1932), is not due to proteolytic action. Cleared milk is coagulated like normal when acidified, and formaldehyde-titration shows no significant increase in amino-N, but rather a decrease of sometimes significant proportions. The organisms seem to assimilate preferentially the free amino-groups of the casein-molecules. The results are seen in Table I. The clearing is probably due to some physical effect upon the casein.

TABLE I.

Action of Mycobacteria and Proactinomycetes in Milk. Inc. 28 days, 28°C.

Organism.	Formol-titrating N, mgm.		Organism.	Formol-titrating N, mgm.	
	Per 10 c.c.	Excess over Control.		Per 10 c.c.	Excess over Control.
Myc. tuberculosis[1]	2·9	(−0·2)	*Myc.* Bb.	2·8	(−0·3)
,, *lacticola*	3·3	(0·2)	,, *equi* aut.	2·8	(−0·3)
,, ,, 37°C.	2·3	−0·8	,, ,, 125	3·3	(0·2)
,, *phlei* aut.	3·2	(0·1)	,, ,, M	2·7	−0·4
,, ,, 282	3·1	0	,, ,, N	3·0	(0·1)
,, *coeliacum* aut.	3·1	0	Glenfield-strain	3·4	(0·3)
,, AI	3·4	(0·3)	*Myc. flavum*	2·8	(−0·3)
,, ,, AIII ..	3·2	(0·1)	*Proact. corallinus*	3·3	(0·2)
,, ,, 18	2·2	−0·9	,, *salmonicolor* ..	1·8	−1·3
,, *rubropertinctum* 279 ..	3·1	0	,, *erythropolis* ..	1·8	−1·3
,, ,, M ..	2·7	−0·4	,, *opacus*	1·9	−1·2
,, ,, 37°C. ..	2·5	−0·6	,, *paraffinae*	2·6	−0·5
,, 272	2·5	−0·6	,, *polychromogenes* ..	4·0	0·9
,,			,, *minimus*	4·6	1·5

All figures are averages of two parallel cultures. 14 titrations of sterile control tubes gave an average of 3·12 ± 0·20 mgm. formol-titrating N per 10 c.c. Differences not exceeding twice the standard deviation are regarded as insignificant, and are placed in brackets in the table.

[1] Incubated at 37°C.

The Genus Corynebacterium.

This genus is much more richly represented in the soil than the mycobacteria (Jensen, 1933). All the organisms studied here conform with the customary definition of the genus. They show constantly the snapping cell division without any slipping. The tendency to formation of branched cells, approaching a mycelium, is rather more pronounced than among the mycobacteria, from which they also differ in a generally much wider range of cell pleomorphism, stronger fermentation of sugars, and generally proteolytic action in milk and gelatine. Many of them invert saccharose and hydrolyze starch, but only one species (*Cor. fimi*) decomposes cellulose. Indole is not formed, although the Salkowski reaction is often positive. Haemolytic effect is very exceptional. All the strains studied here are strictly aerobic, non-motile and grampositive, and they show no acid-

fastness in agar or milk cultures (decolorized by 5% sulphuric acid in 10 seconds). Like Haag (1927), I have found them all incapable of utilizing paraffine, but I cannot confirm Haag's statement that the corynebacteria as a whole require protein as source of nitrogen.

CORYNEBACTERIUM HELVOLUM (Zimmermann) Kisskalt and Berend (1918).

Synonyms: *Bac. helvolus* Zimmermann, cit. after Lehmann and Neumann (1920); *Bact. helvolum* (Zimm.) Lehmann and Neumann (1896–1920); *Flavobacterium helvolum* (Zimm.) Bergey (1923–30). The following organisms are probably identical or closely related: *Bac. citreus* Frankland (1888); Bacterium No. III Düggeli (1904); *Bact. dimorphum* Troili-Petersson (1904); *Bact. Kirchneri* Löhnis (1905). Also the organisms which Greig-Smith (1911) took for rhizobia were probably mostly of this group (Jensen, 1933).

This species-group was the most common soil corynebacterium. Thirteen strains were isolated and studied: A1, A4, N1, N3 from garden soils, B, Bb, C, Ca1, Ca3 from grass soils, 163, 163S, 279 from red soils from Griffith, 121 from sand from Bathurst.

Morphology.—This large species-group shows a wide range of morphological variation. Generally it appears after 16–24 hours at temperatures from 18° to 32°C. as somewhat irregular rods in angular arrangements, occasionally branching; the thickness varies from 0·5 to 1·0μ, the length still more, from 1·0–1·2μ to 10–12μ, according to strain and medium. The rods are generally longer in dextrose-agar than in sugar-free nutrient agar and asparagine-agar, still longer and more branched in potato, especially strain C. The longest cells appear in strain N, the shortest in A1 and 163S. These cell-types are shown in Plate i, figures 11–15. In older cultures the picture becomes still more varied. The simplest cytomorphosis takes place in sugar-free nutrient agar, gelatine, potato and milk-agar, where the cells gradually grow shorter, after 3–4 days appearing largely as cocci of 0·8–1·0μ, in some cases looking exactly like a pure culture of true cocci (Pl. i, fig. 16). In dextrose-agar and asparagine-agar there is after 3–6 days at 28–30°C. (not so much at 18–22°C.) a pronounced tendency to formation of big cystites which in asparagine-agar and other protein-free media are approximately spherical, measuring from 0·8μ up to 3·5μ in diameter (Pl. i, fig. 17); similar cell-types arise in acid dextrose-peptone-solution. Strain C produces no cystites on asparagine-agar, but shows something resembling a division of the rods into cocci and subsequent lateral germination of these into new parallel rods, as described by Haag (1927), a phenomenon which I have not observed in other corynebacteria. In dextrose-agar the cystites are generally first club-shaped, resembling *Cor. diphtheriae*, after 5–6 days uniform thick rods (Pl. i, fig. 20), or spherical to lemon-shaped cells, up to 2·5μ thick. The relative frequency of these cells is variable; in some strains (121, N) they predominate entirely after 5–6 days, but mostly they account for roughly one-half, so that the microscopical picture gives the impression of a mixture of two different organisms (Pl. i, fig. 19); strain C produces no real cystites at 30°C. After 16–20 days the cystites seem gradually to disappear, and the picture is dominated by small cocci, 0·5–0·8μ, often imbedded in amorphous, granular masses. The sediment in milk and broth cultures contains mostly after 3–4 days numerous long, curved and branching rods sometimes resembling small mycelia. Some strains produce large cystites and other irregular cell types, often very long and branching, on dextrose-agar and particularly potato (Pl. i, figs. 22–23) at 37°C.

Similar cystites have been described in other corynebacteria by several authors, as mentioned in the introduction. They might be regarded as gonidangia according to the life-cycle theories of Löhnis (1922) and Enderlein (1925), an idea which is supported by their gradual disappearance and replacement by small cocci imbedded in amorphous masses, which latter might be construed either as remains of the gonidangia or as a symplasm in which gonidia are regenerated. But direct observation of such cystites transferred to various agar media (asparagine-, dextrose or Sabouraud's agar) by means of Ørskov's method (1923) for periods up to 6 days at temperatures from 18 to 30°C. gave different and quite consistent results. If the cystites are not too old, they germinate after one or two days with 1–2 "germ-tubes" and regenerate the slender rods which are characteristic of young cultures (Pl. i, fig. 21). In other cases, especially if the cystites are very big, they remain unchanged until they either seem to dissolve and disappear or are overgrown by neighbour colonies. I have never seen them reproducing their own cell type and thus stabilizing yeast-like forms as contended by de Negri (1916)* and Mellon (1926), but the initial stage of the "germ-tube" appears often as a pear-shaped bud attached with its pointed end to the cystite; this is probably the "budding-discs" of Bergstrand (1923). Neither have I seen any division into four ("tetrads"), as described by Mellon (1926) and Maver (1931); big cystites do, indeed, sometimes in nigrosin-smears, show an appearance suggestive of this, but the phenomenon is not seen in living specimens, and is probably an artefact due to a rupture of the big vacuolized cells during the process of drying. Ørskov (1923) found similar gigantic forms of *Cor. diphtheriae* sometimes capable and sometimes incapable of growth when transferred to fresh media. Several other workers (Stapp and Zycha, 1931; Klieneberger, 1930; Henneberg, 1932) have found such swollen cells in other bacteria incapable of growth when isolated, while the interpretation of these forms as gonidangia by another school of bacteriologists seems based entirely on the study of stained preparations. While not denying the possibility that the cystites examined here may under special conditions develop into gonidangia, we must also conclude that there is at present no positive evidence of this, and the cystites seem simply to represent the "senescent forms" (Henrici, 1928) of the organisms under the conditions at hand. Since not all cells in a culture develop into cystites, it seems likely that those cocci and small rods by which the cystites are gradually superseded, are the old viable "resting" forms of those cells which do not undergo swelling (cf. Klieneberger, 1930).

Cultural characters.—Asparagine-agar: rather scant to good growth, best in strains N and 163, poorest in 163S, restricted, convex, edges even to undulate, surface smooth, glistening, in strain C finely rugose, semi-opaque, soft-pasty, colour ranging from white and cream-coloured to lemon-yellow (in strains A1 and Ca3); strain Bb produces many big opaque secondary colonies after 2–3 weeks; some strains produce a faint dull pink soluble pigment. *Dextrose-agar*: good to abundant growth, slightly spreading, convex, edges even to undulate, surface smooth or slightly folded, glistening, opaque, soft-pasty, colour ranging from cream-coloured or light greyish-yellow to intense chrome-yellow (A1), pigment generally more intense at lower temperature; strain 279 was strongly yellow immediately after

* de Negri found that the big spherical cells generally regenerated the rod-stage when isolated in single-cell culture, until a stabilized "blastomycete" at length was obtained. It is a somewhat suspicious fact that this stabilization should take place only once, and then in a peptone-solution with 10% saccharose—a medium eminently selective for yeasts!

isolation, but lost the pigment after a few transfers. A pale yellowish-brown soluble pigment is often formed. Secondary colonies are common after 3–4 weeks; cultures obtained from these do not seem to differ from the mother culture. Sugar-free nutrient agar gives a similar growth, less vigorous, but often more strongly pigmented. *Sabouraud's agar*: very luxuriant but uncharacteristic growth; some strains (163, N3, Ca1) form a pink soluble pigment. *Potato*: good to abundant growth, somewhat spreading, convex, smooth and glistening, in C and 163 finely rugose during the first 3–6 days, soft-pasty, colour as on dextrose-agar. *Gelatine, plate*: deep colonies spherical with even edge, surface colonies round, convex, smooth, glistening, both finely granular, yellow to transmitted light, with sharply defined opaque central part. *Gelatine, tube*: thin filiform to granular growth in stab; surface colony round, smooth or flatly folded, first convex, later flat and spreading, cream-coloured to chrome-yellow. Liquefaction begins after 6 to 15 days, first saucer-shaped, after 4–6 weeks stratiform, slowest in strains 121 and 163, most rapid in Ca3 and A1. *Broth*: more or less strong uniform turbidity, soft to sticky cream-coloured sediment, occasionally a non-coherent cream-coloured to yellow surface pellicle. *Milk*: soft cream-coloured to yellow surface ring and pellicle, voluminous sediment of the same colour; no coagulation at 28–30°C., but gradual digestion in 2–5 weeks (most rapid in strains B, Ca1 and Ca3), with neutral to alkaline reaction. At 37°C. most strains produce a soft coagulation after 2–4 weeks. *Milk-agar*: growth fair to very abundant, white to pale yellow; some strains (Ca1, N3) form a pink pigment; proteolytic zones are very clear and broad (8–12 mm. after 4 days) in strains B and C, narrower in the others, but always present.

Physiological characters.—Nitrate, ammonia and asparagine are utilized (best by N3), although they are inferior to peptone. Nitrate is reduced to nitrite by most strains. All except A1 invert saccharose. All except B and 163S hydrolyze starch, 121, Bb and N3 strongly. All 13 strains produce acid from dextrose, glycerine and mannite, 12 from arabinose, 11 from saccharose, 9 from galactose, 7 from levulose, 6 from lactose, 3 from maltose, none from dulcite. The acid reaction is sometimes transient and changes to alkaline after 12–14 days. No correlation was noticed between vigour of growth and intensity of acid-production. All strains are proteolytic in milk, but to a different degree (Table 2). Optimal reaction is approximately pH 6·2–7·2; growth stops mostly at pH 4·3–4·6. Dextrose-NH_4Cl-solution is acidified to pH 3·9–4·6. Optimal temperature seems to be about 28–32°C.; most strains grow quite well at 37°C., some scantily.

Although the strains vary considerably, there is no discernible correlation between the variations, and no subgroups can be distinguished; we must therefore regard all 13 strains as one species-group.

Dissociation.—Two types of variants were produced.

i. "*Slimy variants*" were obtained of strain 121 from 102-days-old culture in dextrose-peptone-solution of pH 6·8, of Ca1 from 202-days-old culture in lithium-solution and 164-days-old culture in uranium-solution, and of N3 from 68-days-old culture in uranium-solution. These variants resembled generally the strains from which they had been derived, but differed in two respects. In 2–7-days-old cultures on solid substrata, especially potato and Sabouraud's agar, they produce a moist, somewhat transparent, extremely viscid growth of an almost glue-like consistence; older cultures are opaque and non-viscid, but become so on transfer. Morphologically they are shorter and plumper than the "parental" strains (the 121-variant almost coccoid), with less branching and less tendency to cystite

formation. The 121-variant reverted to a non-viscid type after 130 days' growth in broth; the reverted form remained shorter and plumper than the original. Strain 163 showed viscid colonies in platings from 90–110-days-old cultures in dextrose-peptone-solution of pH 4·6–7·2, but transfers from these colonies proved non-viscid in the first subculture.

ii. "*Myceloid variants*" were obtained of strain 163 from 150-days-old culture in lithium-solution, and of N3 and Bb respectively from 107- and 118-days-old broth cultures. These variants produce a somewhat spreading, flat, lobate and folded growth in agar media, and a finely and strongly wrinkled growth in potato after 2–5 days, later becoming smooth and glistening. Their cells in young agar cultures are longer and more richly branching than those of the "parental" strains, which they otherwise resemble, and show numerous granules of aerial "mycelium". On potato they, especially the N3-variant, produce actual mycelia after 1 day (Pl. i, figs. 24–25), with numerous branches arising as small lateral pear-shaped knobs or buds which gradually stretch into filaments, as in the actinomycetes (Jensen, 1932). With advancing age the mycelia divide into rods of varying length, and in dextrose-agar they often produce cystites of sometimes enormous dimensions (Pl. ii, fig. 27); these appear highly vacuolized, stain badly, and contain generally one or two refractive granules which show a lively Brownian movement (cf. Bergstrand, 1918). Such large cystites have regularly failed to grow when transferred to fresh agar. In old cultures they seem to disintegrate, and small short rods and cocci remain between their residues, thereby giving a picture which might well be interpreted as "liberation of gonidia" or "regeneration of cells from symplasm" (Pl. ii, fig. 28). The buds which represent the initial stages of the mycelial branches look exactly like the "regenerative bodies" of Löhnis (1922), and may conceivably function as such if detached from the parent cell, but there is no indication that this plays a special rôle as a mode of reproduction. The original strain C appears similar to the myceloid variants, to judge by its cultural appearance and its morphology in young potato cultures (Pl. i, fig. 15). Finally it is to be mentioned that the myceloid variants show little branching in sugar-free nutrient agar, where they appear like the original strains.

A variant of A4 was obtained from uranium-solution, showing a spreading, folded and wrinkled growth in agar media, but hardly any indication of mycelial growth. A somewhat similar form of 279 was obtained from lithium-solution.

CORYNEBACTERIUM CREMOIDES Lehmann and Neumann (1927)?

Synonym: *Bacterium cremoides* Lehmann and Neumann (1911–20). *Bact. cocciforme* Migula (1900) seems related.

Two strains, from garden soil (A) and grass soil (C). The strains are closely related to *Cor. helvolum*, and differ from Lehmann and Neumann's description in being slightly proteolytic.

Morphology.—Similar to *Cor. helvolum*, but shorter, 0·5–0·8μ × 2–3μ, and with less tendency to formation of cystites; both strains produce cocci of a diameter up to 2·5μ in asparagine-agar after 8–10 days, strain A also in dextrose-agar at 37°C. Strain C formed, immediately after its isolation, many big spherical cystites, up to 3·5μ in diameter, in broth culture. They failed to grow when transplanted to agar, and disappeared in the second subculture.

Cultural and physiological characters are mainly as in *Cor. helvolum*. The colour of the growth is creamy to greyish-yellow, never pure yellow. Liquefaction of gelatine and digestion of milk are definitely slower, not noticeable until after

4–5 weeks; milk is softly coagulated after 2–3 weeks at 37°C. Strain A reduces nitrate to nitrite and inverts saccharose. Both strains produce acid in dextrose, glycerine and mannite, A also in levulose, galactose and saccharose. This strain is capable of a faint growth at pH 4·3.

Dissociation.—A "slimy" variant of A was obtained from 235-days-old culture in lithium-solution; it was, like the slimy variants of *Cor. helvolum*, strongly viscid in young cultures, and morphologically somewhat shorter and plumper than the original strain.

CORYNEBACTERIUM INSIDIOSUM (McCulloch), n. comb.

Synonyms: *Aplanobacter insidiosum* McCulloch (see Jones and McCulloch, 1926); *Erwinia insidiosa* (McCulloch) Bergey (1930).

One strain, isolated from grass soil.

Morphology.—Almost identical with *Cor. helvolum*; in 1-day-old agar and potato cultures irregular rods in angular position, 0·5–0·7μ × 2–6μ. Beautiful club-shaped rods in dextrose-agar and milk-agar after 3–6 days, spherical cystites, 2·5–3·0μ, in asparagine-agar after 8 days. In sugar-free nutrient agar only small slender rods, 0·4–0·6μ × 0·8–1·5μ.

Cultural characters.—*Asparagine-agar*: good growth, restricted, convex, edges undulate, surface smooth, glistening, opaque, white, becoming cream-coloured. *Dextrose-agar*: abundant growth, restricted, convex, edges even, surface smooth, glistening, opaque, cream-coloured, becoming greyish-yellow. The organism showed after a few transfers an increasing production of a blue-violet insoluble pigment appearing as streaks in the growth, particularly near the edge. This pigment is formed most copiously at low temperatures (16–18°C.), and appears microscopically as roughly spherical, deep blue granules of 2–8μ diameter, exactly as described by Jones and McCulloch (1926). In sugar-free nutrient agar the growth is less vigorous, cream-coloured without blue pigment. *Potato*: good growth, spreading, convex, smoothly folded, opaque, greyish-yellow. *Gelatine*: thin filiform growth in stab; round, convex, smooth, cream-coloured surface colony; very slow saccate liquefaction, starting after 4–5 weeks. *Broth*: faint uniform turbidity, later clear with soft cream-coloured sediment. *Milk*: cream-coloured sediment and pellicle, slow digestion without coagulation; reaction neutral. *Milk-agar*: thin white growth, very slight proteolysis.

Physiological characters.—Nitrate, ammonia and peptone are readily utilized. Reduction of nitrate to nitrite, faint. Saccharose is inverted. Diastatic action faint. Acid is produced from arabinose, dextrose, levulose, saccharose, glycerine, mannite, and dulcite. Proteolytic action in milk weaker than that of *Cor. helvolum* (Table 2). Optimal reaction, pH 5·6–6·8; growth stops at pH 4·3–4·6. Dextrose-NH_4Cl-solution is acidified to pH 4·0–4·1. No growth, or only a trace, at 37°C.

Dissociation.—A "slimy" variant was isolated from 97-days-old culture in dextrose-peptone-solution of pH 7·2. It produces an abundant, moist and fluid, very viscid growth on agar and potato, and forms the characteristic blue pigment on dextrose-agar. Its cells are rather smaller and more slender than those of the original type, with no tendency to formation of cystites in dextrose-agar, where only short rods and small cocci are seen.

The slimy variant seems to agree better than the original type with Jones and McCulloch's description of their organism which was pathogenic to lucerne plants, produced a viscid growth in agar, and did not vary much in its morphology in different media. Their careful description and instructive microphotographs leave no doubt that the organism was really a corynebacterium. The present

strain seems to differ from it only in a few minor points: more vigorous growth, less definite yellow pigment in nutrient agar, no blue pigment on potato, no coagulation of milk, higher temperature maximum, and more resistance to acid reaction. An infection experiment did not show the organism to be pathogenic to lucerne.* We may therefore call this strain *Cor. insidiosum* var. *saprophyticum*.

CORYNEBACTERIUM FILAMENTOSUM, n. sp.

Five strains, 163, 272a, 272b, 276, 279, isolated from red soils from Griffith; the last strain died out during the work.

Morphology.—This characteristic species shows considerable variation according to the medium. 1–2-days-old cells in asparagine-agar are rod-shaped and typically curved, vibrio-like, mostly 0·5–0·8$\mu \times$ 2–7μ, sometimes longer and branched, always in very striking parallel bundles (Pl. ii, fig. 29). Living cells, which multiply by typical angular growth, exhibit alternating bands of more and less refractive parts of the protoplasm, which in connection with the angular and parallel arrangement of the cells give the quite young colonies on asparagine-agar a most characteristic patterned look when seen under a high-power dry lens, something like a pleat-work of straw; later the thin, flat colonies with their lobate edges look like ice-plants. The cells of most strains grow shorter (1·0–4·0μ) in this medium after 4–7 days; strain 279 showed many long branching cells after 5 days (Pl. ii, fig. 31), later belt-staining and ghost-forms. At 37°C. there are, in strain 272b after 3 days, only slender rods, in the others numerous fusiform to club-shaped cells up to 1·2μ thick. In dextrose-agar and Sabouraud's agar all strains produce, after 1–2 days, long, curved and wavy, often branched filaments 0·8–1·0μ thick (Pl. ii, fig. 30), which in older cultures either break up into shorter fragments or remain as long, thick, irregular, twisted filaments, badly staining or with marked belt-staining, 1·2–1·5μ thick. Similar forms arise in sugar-free nutrient agar, but the long filaments are less numerous here. In some cultures of this species, a few cells exhibit peculiar oscillatory or rotatory movements, but do not show any actual locomotion (cf. Jones and McCulloch, 1926).

Cultural characters.—*Asparagine-agar*: good and very characteristic growth, widely spreading, central part convex, smooth, glistening, white, sending raised dendritic projections into the broad marginal part, which is flat, dull, finely rugose, with lobate edges, white, bluish to transmitted light; strain 272a lacks this characteristic margin, and produces a restricted growth with even edges. Most strains (except 276) produce a light greenish-yellow soluble pigment. *Dextrose-agar*: growth much less vigorous than on previous, strain 163 no growth at all, the others scant to fair, 276 best, narrow, flat to slightly convex, edges undulate, surface smooth to slightly folded, cream-coloured to greyish-yellow, somewhat viscid. The growth in sugar-free nutrient agar and Sabouraud's agar is similar, rather weaker; strain 163 produces a faint growth in these media. *Potato*: scant growth, 163 none, narrow, flat, glistening, cream-coloured to greyish-yellow or almost grey, surrounded by a white halo, in 276 becoming raised and folded; consistence gum-like. *Gelatine*: plate colonies very small, spherical, edges smooth, interior finely granular with opaque centre. Tube: thin filiform white growth in stab; small, round to lobate, white to cream-coloured surface colony; liquefaction slow, starting in about a week, first funnel-shaped to saccate, later stratiform. *Broth*: faint uniform turbidity, small soft flaky cream-coloured sediment; strain

* My best thanks are due to Dr. W. L. Waterhouse, of the Faculty of Agriculture, University of Sydney, for assistance in carrying out this experiment.

163 does not grow. *Milk*: white to cream-coloured surface ring and sediment; no coagulation. Digestion in 2–4 weeks, most slowly by 163; reaction neutral to faintly acid. *Milk-agar*: abundant, opaque, spreading, white to cream-coloured growth, surrounded by clear proteolytic zones 10–12 mm. broad in 7 days.

Physiological characters.—Ammonia is utilized as readily as peptone, and asparagine still better; nitrate is not utilized by strains 272b and 279, readily by the others. Strains 163 and 272a reduce nitrate to nitrite. Saccharose is not inverted; starch is not hydrolyzed. Strain 276 produces acid from glycerine and, to a slight extent, arabinose; in all other cases there is a strong and rapid alkali-formation in the sugar-media. Proteolytic action in milk varies from weak to very strong (Table 2). Optimal reaction, pH 6·8–7·2; growth stops at pH 5·3–5·6. Dextrose-NH_4Cl-solution is acidified to pH 5·4–5·5, by 276 to pH 5·0–5·1. The group is more sensitive to acid reaction than most saprophytic corynebacteria. Excellent growth at 37°C.

Dissociation.—Strain 276 produced, after 124 days' growth in dextrose-peptone-solution of pH 6·8–7·2, a kind of "smooth" variant which had lost the characteristic flat dull fringe on asparagine-agar and appeared like strain 272*a*, from which it differed in a better growth in dextrose-agar and in not forming yellow soluble pigment. Morphologically it was like the original. In 90–100-days-old cultures it produced on dextrose-agar small opaque greenish-grey secondary colonies. Plating from these on asparagine-agar yielded two types of colonies: (*a*) like the mother-culture, and (*b*) much smaller, in subculture appearing as a dwarf-form of the variant. This was the only case in the whole investigation where a secondary variant was found. Its cells on asparagine-agar after 1 day are very small, slender, curved, 0·3–0·4μ × 1·2–2·5μ, in dextrose-agar straight, up to 4μ long, but after 3 days only 1·0–1·5μ. In Sabouraud's agar it forms after 3 days irregular curved and sometimes branching rods of varying thickness, 0·3–0·8μ × 1·0–7·0μ, some with club-like swellings. Culturally it is like the primary variant, but does not grow quite so vigorously, and produces yellow pigment in asparagine-agar, where its young colonies have the characteristic patterned appearance.

This species-group seems closely related to the "*Vibrio*" *linguale* (Weibel) Migula (1900), an organism which Bajardi (1905) considered a "streptothrix", as well as to *Bact. racemosum* Zettnow (1915), but according to the descriptions it is hardly identical with any of these. If this group of organisms is to be regarded as a separate genus, its name will be *Zettnowia* Enderlein (1925).

Corynebacterium simplex, n. sp.

Two strains, from grass soil (B) and from red soil from Griffith (282).

Morphology.—It appears after 1–2 days on asparagine-agar much like *Cor. filamentosum*, to which it is closely related. The rods are somewhat shorter and more slender, 0·4–0·5μ × 3–5μ, curved and in parallel bundles (Pl. ii, fig. 32). Young colonies on asparagine-agar show microscopically the characteristic patterned appearance. In older cultures no branching is seen, but the cells grow shorter, almost coccoid. The species is well distinguished from the previous by the absence of long branching filaments in dextrose-agar and Sabouraud's agar, where it appears after 1–4 days as straight, slender rods, 0·4–0·6μ × 2–4μ, in angular arrangement (Pl. ii, fig. 33). In sugar-free nutrient agar it produces after 5–10 days minute rods and cocci, 0·3–0·4μ × 0·5–1·5μ. Strain B produces in dextrose-agar at 37°C. some swollen cuneate rods and cocci up to 1·5μ thick; otherwise cystites are generally absent in this species.

Cultural characters.—*Asparagine-agar*: fair to good growth, very similar to *Cor. filamentosum*, with less broad marginal part, and becoming all moist and glistening with advancing age; no pigment. *Dextrose-agar*: abundant growth, spreading, slightly convex, edges even, surface smooth, glistening, cream-coloured to greyish-yellow, soft-pasty. Sugar-free nutrient agar similar, not so abundant growth, in strain B small opaque greenish-grey secondary colonies after 6 weeks. *Potato*: good growth, spreading, flat, smooth, very moist, greyish-white, 282 becoming greyish-brown; strain B slimy growth. *Gelatine*: plate colonies very small, spherical, with granular edge and interior. Tube: thin filiform growth in stab; small round smooth cream-coloured surface colony; liquefaction starts after 4 days, first saucer-shaped, later stratiform. *Broth*: uniform turbidity, small white sediment, later greyish-yellow and viscid. *Milk*: yellowish ring around surface, in B slimy white pellicle after 2 weeks. No coagulation; perfect digestion in 10–12 days; reaction neutral. *Milk-agar*: abundant white growth, becoming cream-coloured; clear proteolytic zones, 12–18 mm. broad in 7 days. Strain B produces much slime in various nutrient solutions.

Physiological characters.—Nitrate, ammonia and asparagine are readily utilized, the last as readily as peptone. Nitrate is reduced to nitrite. Strain 282 seems to invert saccharose. Starch is not hydrolyzed. Slight production of acid from saccharose in old cultures (14–20 days), else a rapid alkali-formation. This is the most proteolytic corynebacterium examined (Table 2). Optimal reaction, pH 6·8–7·2. Growth stops at pH 4·9–5·3. Dextrose-NH_4Cl-solution is acidified to pH 4·5–4·9. Excellent growth at 37°C.

Dissociation.—Plating from 142-days-old culture of strain B in lithium-solution yielded a dwarf-form appearing in two types: a "rough" and a "smooth". Both produced a scant white growth in asparagine-agar, narrow, dull and rugose in the "rough", more spreading, moist and glistening in the "smooth". In dextrose-agar good growth, flat, smooth, glistening, cream-coloured, the "smooth" more spreading. In broth uniform turbidity, the "rough" producing a coherent pellicle after 10–12 days. Microscopically the "smooth" type appears in asparagine-agar after one day as minute curved rods, 0·3–0·5μ × 1·0–1·5μ, in parallel bundles (Pl. ii, fig. 34), the "rough" more irregular, sometimes branching, straight, with many granules of aerial "mycelium", after 2 days producing burr-shaped colonies like the mycobacteria. After 6–18 days both types appear similar, as short, plump, almost coccoid rods, 0·6–0·8μ × 1·0–1·2μ. The colonies of both types have the characteristic ice-plant-like appearance. In dextrose-agar they are similar, after one day short, somewhat curved rods, 0·4–0·5μ × 1·5–2·5μ, gradually growing shorter and plumper, after 18 days almost coccoid, 0·6–0·8μ. This variant bears no small resemblance to the secondary variant of *Cor. filamentosum*, which by its lack of long filaments in dextrose-agar seems to stand as a kind of transition to *Cor. simplex*.

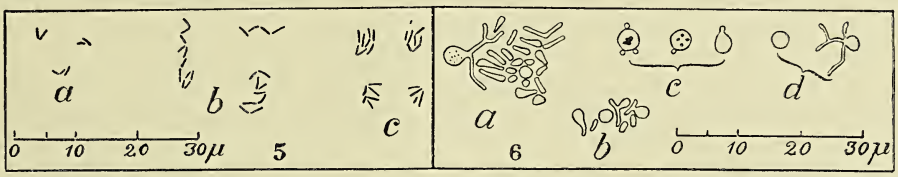
CORYNEBACTERIUM NUBILUM (Frankland), n. comb.

Synonyms: *Bacillus nubilus* Frankland, cit. after Bergey (1930); *Bacterium nubilum* (Fr.) Lehmann and Neumann (1911–27); *Flavobacterium nubilum* (Fr.) Bergey (1930).

The present single strain, isolated from garden soil, appears to be a small and feebly growing variety of the form which Lehmann and Neumann (1927) mention as possibly a corynebacterium. It forms no yellow pigment and has no connection with *Flavobacterium* as defined by Bergey. The name *Cor. nubilum* var. *nanum* might be suggested for this particular variety.

Morphology.—The organism has little in common with other corynebacteria. It appears as a minute rod, not much bigger than the influenza bacillus, mostly 0·3–0·4μ × 0·8–1·0μ, not varying much in different media or at different temperatures or ages. Longer rods, up to 5μ, may be seen in dextrose-agar and Sabouraud's agar, but branched and club-shaped cells do not occur. The cells stain badly and appear in typical V-figures and broad parallel bundles. A very distinct angular growth takes place in asparagine-agar (Text-fig. 5). This, in connection with its being non-motile, non-spore-forming and grampositive, is the main reason why it has been here included in *Corynebacterium.*

Cultural characters.—The organism grows slowly and feebly in all media; the growth became somewhat better and more rapid after prolonged cultivation. *Asparagine-agar*: scant growth, spreading, flat, thin, edges lobate, surface finely rugose, at first dry and dull, later moist and glistening, white, resembling *Cor. filamentosum* and *Cor. simplex. Dextrose-agar*: slow, but eventually fair growth, spreading, flat, dull, colourless with pink centre, after 3 weeks moist and glistening, pale pink. *Sabouraud's agar*: like previous, but better growth, dull red after 12 days. *Potato*: no growth. *Gelatine*: thin, granular, pink growth in stab; small red wrinkled surface colony; liquefaction very slow, at first saccate, after 3–4 months stratiform. *Broth*: faint uniform turbidity, becoming quite strong after 2 weeks; no pellicle or sediment. *Milk*: no growth.



Text-figure 5.—*Cor. nubilum.* Direct agar-microscopy, asparagine-agar, 15-16°C. *a*, 3 days; *b*, 6 days; *c*, 9 days.

Text-figure 6.—*Cor. tumescens. a-b.* Strain A, dextrose-agar, 3 days, 28-30°C.; *c.* Strain A, milk-agar, 10 days, 28°C.; *d.* Strain B, cystite from Sabouraud's agar, 10 days, 28°C., germinating after 4 days on same medium, 20-22°C.—*a, b, d,* direct agar-microscopy; *c,* unstained wet mount.

Physiological characters.—Ammonia and peptone are utilized feebly, asparagine slightly better, nitrate not at all; the compounds in meat-extract and Sabouraud's agar seem essential for a good growth. Nitrate is not reduced. Saccharose is not inverted. Starch is not hydrolyzed. No acid from carbohydrates. No proteolytic action in milk, but apparently in gelatine. Growth at 37°C. seems better than at 30°C.

Corynebacterium tumescens, n. sp.

Three strains: A from garden soil, B and 18 from grass soils.

Morphology.—This species shows a most characteristic cytomorphosis in dextrose-agar, Sabouraud's agar and milk-agar. Cells in dextrose-agar after 18–24 hours at 28–30°C. are curved, often branched, show angular arrangement (Pl. ii, fig. 36), 0·5–0·8μ × 2·5–6μ, in B shorter and less branched. After 2–3 days many spherical to club-shaped cystites, up to 3μ thick, arise as local swellings of the rods (Text-fig. 6). At first staining intensely, they gradually change into big, irregular, badly staining "ghost forms" which contain many deeply-staining belts and granules. Besides these one notices irregular, less swollen, intensely staining rods and small, intensely staining cocci, 0·4–0·5μ, which resemble the granules

present in the cystites. These cocci prove to be alive and capable of developing into rods when transferred to fresh media. In milk-agar the big coccoid cystites are often found in almost pure culture (Pl. ii, fig. 40); they have sometimes 2–4 small cocci attached to the outer cell wall, thereby giving a picture like that of a budding yeast cell (Text-fig. 6). When transferred to fresh agar, the cystites either fail to develop altogether, or (especially in strain B) germinate with the formation of one or two, occasionally three or even four slender sprouts which regenerate the rods present in young cultures (Pl. ii, fig. 41). This is the course of development when isolated cystites are observed. When cystite-material is put on fresh agar without "spreading" and examined after 16–24 hours, one finds a large number of small cocci which appear to sprout from the cystites (Pl. ii, fig. 39), and which resemble the cocci described above. It seems likely that those elements which regenerate the slender rods from isolated cystites, may in mass culture break off from the cystites as cocci, before developing into rods, and function as gonidia. This is the only species of corynebacteria studied by me which has shown some positive evidence of reproduction by gonidia. The cystites are formed most abundantly at 30–37°C.; at 16–18°C. they are sometimes not noticeable at all (Pl. ii, fig. 37). In old cultures on Sabouraud's agar they sometimes reach a diameter of 6–8μ (Pl. ii, fig. 38). They are also formed in nutrient agar, but in media poor in nutrients, such as the dextrose-casein-agar used for the isolation of the corynebacteria, no cystites are seen. The organism appears here after one day as long slender rods which rapidly grow shorter and after 3–4 days appear as cocci of 0·5–0·6μ diameter (Pl. ii, figs. 42–43).

Cultural characters.—*Asparagine-agar*: very scant growth, narrow, thin, flat, moist, colourless streak. *Dextrose-agar*: fair growth, restricted, convex, edges even, surface smooth, glistening, white to cream-coloured, semi-transparent. *Sabouraud's agar*: good growth, slightly spreading, convex, edges lobate, surface smooth, glistening, cream-coloured, in strain B greyish-pink; a pink soluble pigment is sometimes formed. *Potato*: slow, but eventually good growth, restricted, convex, smooth, glistening, greyish-white to dirty cream-coloured, in B greyish-orange; consistence strongly viscid. *Gelatine*: plate colonies very small, spherical, edges smooth, interior granular, opaque, yellow. In stab thin white filiform to granular growth, very small white surface colony; liquefaction very slow, starts after 3–4 weeks, funnel-shaped. *Broth*: faint uniform turbidity, after 2–3 weeks a soft white to cream-coloured sediment. *Milk*: thin white ring around the surface; soft coagulation after 18–20 days, later slow digestion; reaction faintly acid. *Milk-agar*: good growth, opaque, in 18 and A white, with 4–5 mm. broad proteolytic zones in 14 days, in B with pink tinge and very slight clearing.

Physiological characters.—Nitrate, ammonia and asparagine are but very slightly utilized. Nitrate is reduced to nitrite. Strain 18 inverts saccharose. Starch is hydrolyzed faintly by strain B. Acid is produced from arabinose, dextrose, galactose, maltose and glycerine by all strains, from saccharose by 18 and B, and from mannite by A and B. Weak proteolytic action in milk (Table 2). Strain B grows well at 37°C., the others more scantily than at 28–30°C. Optimal reaction, pH 6·2–6·8. Growth stops at pH 4·9–5·3.

Dissociation.—Strains 18 and B produced "slimy" variants after 172 days' growth in lithium-solution; B produced also such a variant after 3 passages on a similar agar-medium. The variants differ morphologically from the original strains in a less pronounced tendency (especially the 18-variant) to cystite-formation in dextrose-agar and Sabouraud's agar, where they appear after 3–6

days as irregular, curved, branching and club-shaped rods and filaments of varying length (Pl. ii, fig. 44). The growth on agar is, especially in the B-variant, of an extremely viscid consistence. Otherwise they resemble the original types, and the B-variant shows a still stronger production of pinkish-orange pigment.

CORYNEBACTERIUM MICHIGANENSE (Smith), n. comb.

Synonyms: *Aplanobacter michiganense* Smith (1914); *Erwinia michiganense* Bergey (1930); *Bacterium michiganense* (Sm.) Stapp (1930).

This species has been carefully examined by Stapp (1930), whose fine micro-photographs and generally good description leave no doubt that it is really a corynebacterium. The same is evidently true of the closely related *Bact. sepedonicum* (Stapp, 1930) and *Aplanobacter rathayi* (Smith, 1914).

The authentic culture was compared with a strain of very similar appearance, isolated from grass soil.

Morphology.—Both strains appear after one day at 28–30°C. in nutrient agar with or without dextrose, Sabouraud's agar, potato-extract agar and potato as small, straight, slender rods in typical arrangement, 0·4–0·6μ × 1·2–3·0μ; the authentic strain exceeds rarely 0·5μ in width and 2·0μ in length. Direct agar-microscopy shows distinct angular growth. The soil strain produces a few branching cells on Sabouraud's agar after 18 hours at 16°C., and the authentic strain becomes somewhat bigger and plumper, 0·5–1·0μ × 1·2–2·5μ, in Sabouraud's agar and dextrose-agar after 7 days at 28–30°C. This seems to be an instance of "mature forms" being bigger than "embryonic forms" (cf. Henrici, 1928), a phenomenon which I have not found generally true of the corynebacteria. The soil strain appears in dextrose-agar after one day at 37°C. as long, irregular, branching, club-shaped rods, exactly resembling *Cor. diphtheriae* (Pl. ii, fig. 35). These clubs develop after 3–5 days into spherical to pear-shaped cystites, 2·0–2·5μ thick. When transplanted to fresh agar, the real cystites constantly fail to grow, whereas less strongly swollen cells may multiply, in which case they regenerate the normal rods. The authentic strain produces no visible growth at 37°C., but the cells in the inoculum appear somewhat swollen and club-shaped after 18–24 hours at this temperature.

Cultural characters.—*Asparagine-agar*: trace of growth only; narrow, thin, colourless streak. *Dextrose-agar*: good growth, restricted, convex, edges even, surface smooth, glistening, white to cream-coloured, authentic strain with yellowish-brown centre after 2 weeks. In sugar-free nutrient agar the growth is fair only, somewhat folded, light ochre-yellow. *Sabouraud's agar*: abundant growth, authentic strain restricted, convex, with even edges and smooth surface, soil strain widely spreading, moist and fluid, both viscid, ochre-yellow, becoming almost sepia-brown after 2–3 weeks. *Potato*: abundant growth, spreading, flat, smooth, glistening, viscid, deep ochre-yellow; potato grey. *Gelatine*: granular yellow growth in stab; small wrinkled ochre-yellow surface colony; liquefaction very slow, saucer-shaped after 4–5 weeks. *Broth*: faint turbidity, after 2–4 weeks clear with small cream-coloured to light yellow sediment, sand-like in the authentic strain, slimy in the soil strain. *Milk*: small yellow sediment and surface ring; the soil strain coagulates the milk firmly after 10 days, the authentic more softly after 20 days. Slow digestion; reaction definitely acid. *Milk-agar*: dense ochre-yellow growth; the soil strain produces a fairly rapid clearing (4–6 mm. broad zones in 7 days), the authentic almost none.

Physiological characters.—Nitrate and ammonia are hardly utilized, asparagine very imperfectly. Nitrate is not reduced to nitrite. Saccharose is slightly inverted.

Diastatic action doubtful. Both strains produce acid from all the carbon-compounds tested, with the exception of dulcite, where the soil strain also gives a doubtful reaction; the acid-production is stronger and more rapid in this than in the authentic strain. The soil strain shows a faint haemolytic effect and is moderately proteolytic in milk. Its optimal reaction is pH 6·2–6·8, but it still continues to grow at pH 4·3. Scant growth at 37°C.

Since the soil strain seems to differ from the authentic only in its more rapid and moist growth, its higher temperature maximum and its stronger proteolytic activity, we may regard it as a *Cor. michiganense* var. *saprophyticum*.

The descriptions of *Bact. fulvum* (Zimmermann) by Migula (1900) and Lehmann and Neumann (1920) are not unlike the present soil strain. Haag (1927) mentions that the *Bact. fulvum* in the collection of the Hygienic Institute of Würzburg utilizes paraffine and appears like *Myc. phlei* in subculture herefrom; Lehmann and Neumann (1927) mention it as a corynebacterium. The form of *Bact. fulvum* examined here (from the Lister Institute) proved to be a simple, non-motile rod without any branching or club-formation, but growing in long chains without any indication of slipping or angular growth; paraffine was not utilized, nor did the organism show any other resemblance to the mycobacteria or corynebacteria. The London strain is thus obviously different from the Würzburg strain, and it seems hardly possible to see which of them is identical with Zimmermann's original organism.

CORYNEBACTERIUM FIMI (McBeth and Scales), n. comb.

Synonyms: *Bacterium fimi* McBeth and Scales (1913); *Cellulomonas fimi* (McB. and Sc.) Bergey (1923–30). Only the authentic strain examined here.

Morphology.—McBeth and Scales' microphotograph shows a certain "diphtheroid" appearance, and this was confirmed by direct agar-microscopy, which shows a typical angular growth. The organism appears on nutrient agar after 18–24 hours as a small straight rod, largely in V-figures, 0·4–0·5μ × 1·2–2·5μ, after 5–15 days (and at 37°C.) shorter, 1·0–1·5μ, otherwise not changing much. In dextrose-agar and Sabouraud's agar the cells are, after one day, longer, curved and of a striking "diphtheroid" type (Pl. ii, fig. 47). Many longer, irregular, curved, club-shaped and branching cells, up to 9μ long, are formed in these media as well as on potato after 5–21 days. The gram-reaction of the organism is variable; McBeth and Scales report it as gram-negative. It seems to be gram-labile like certain other corynebacteria, e.g., *Cor. pyogenes* (Brown and Orcutt, 1920).

Cultural characters.—*Asparagine-agar*: very scant growth, narrow, thin, glistening, white. *Nutrient agar*: restricted, convex, fair growth, smooth, glistening, opaque, lemon-yellow. On dextrose-agar and Sabouraud's agar the growth is more scant, cream-coloured. *Potato*: slow, but after 2–3 weeks good growth, raised, folded, glistening, chrome-yellow. *Gelatine*: granular yellow growth in stab; small round yellow surface colony, becoming lobate; liquefaction slow, saucer-shaped, starting after 10–14 days. *Broth*: uniform turbidity, strong after 3 weeks; small soft cream-coloured to yellow sediment. *Milk*: small yellow sediment; coagulation after 3 weeks at 37°C. (none at 28–30°C.); reaction acid.

Physiological characters.—Nitrate, ammonia and asparagine do not seem to be utilized. Nitrate is reduced to nitrite. Saccharose is not inverted. Diastatic action doubtful. Acid is produced from all the carbon-compounds tested, but only feebly from mannite and dulcite. Better growth at 37°C. than at 28–30°C. This

species alone of all the organisms examined here caused a rapid disintegration of cellulose supplied as filter paper in a 0·5% peptone-solution.

The morphology of the organism shows plainly that it belongs to the corynebacteria, and its ability to decompose cellulose would hardly be a valid reason to include it in the genus *Cellulomonas* together with morphologically different organisms. The same is probably true of the closely related *Bact. liquatum* McBeth and Scales (1913).

The Corynebacterium liquefaciens-Group

Type species: *Corynebacterium liquefaciens* (Orla-Jensen), n. comb. Synonym: *Microbacterium liquefaciens* Orla-Jensen (1919).

This group attaches itself closely to *Cor. fimi*. The strains, although similar to each other, are too different to be united into a single species, and since no two strains agree well, I have refrained from naming any new species. *Cor. flavum* and *Cor. pyogenes** Berend and Kisskalt (1918) and the serum-liquefying form isolated from oyster by Barratt (1924) are probably related to this group, as are also possibly some of the bacteria described by Townsend (1929), among which Group I appears to be corynebacteria.

The group is represented by 5 soil strains (B2, B3, 11, 18, 276) and *Micr. liquefaciens* Orla-Jensen. The last organism shows a distinct angular growth (as may also be seen from Orla-Jensen's microphotograph) and is in every other respect a true corynebacterium.

Morphology.—All strains are fairly uniform in 18–24-hours-old cultures on dextrose-agar, Sabouraud's agar and potato; slender, often curved and bent rods, mostly 0·4–0·6μ × 1·2–5·0μ, in typical angular arrangements (Pl. ii, fig. 45), all exhibiting angular growth by direct agar-microscopy. In older cultures the rods grow shorter, almost coccoid to pear-shaped (Pl. ii, fig. 46), sometimes increased in thickness to 0·7–0·8μ, but without typical cystites. Branching occurs in young stages, but not frequently. Strain 276 shows in Sabouraud's agar after 18–20 days remarkable long sinuous filaments, sometimes with terminal swellings 0·8–1·0μ thick, not unlike the "spirochaetoid" forms of diphtheroids described by Bergstrand (1919). Otherwise this group of corynebacteria, like *Cor. fimi*, shows less morphological differentiation than the others.

Cultural characters.—All strains produce a mere trace of growth in asparagine-agar. In dextrose-agar the growth is fair, soft and smooth, cream-coloured, in strain 11 quite scant, light yellow. A better growth takes place in Sabouraud's agar, often intense chrome-yellow to greenish-yellow. Strain 11 shows the remarkable property of growing well only in agar which has previously supported growth of acid-fast organisms (*Myc. lacticola, Myc. rubropertinctum, Proact. corallinus*) for 2–3 days and then been re-sterilized. Growth on potato is mostly scant to fair, cream-coloured to lemon-yellow, in strain B2 abundant, intense yellow. The growth in gelatine is mostly scant and not very characteristic, except in strain 18, which produces a thick, wrinkled, spreading, intensely chrome-yellow surface layer; all strains, with the exception of B2, liquefy the gelatine slowly. *Cor. liquefaciens*, B3 and 276 cause a soft coagulation of milk after 3–4 weeks; digestion is either very slow or not visible at all.

Physiological characters.—Non-protein N-compounds are not utilized to any significant extent. *Cor. liquefaciens* and 18 reduce nitrate to nitrite. B2, 11 and

* The latter name is hardly valid, since it appears to belong to another corynebacterium (Brown and Orcutt, 1920).

276 invert saccharose. B2 and 276 hydrolyze starch feebly. Acid-production is variable; the extremes are represented by 276, which produces acid from all carbon-compounds except lactose, and by B3 which ferments only dextrose and galactose. Proteolytic action in milk is weak or absent; strain B2 is the only one of the whole series which seems non-proteolytic both in milk and in gelatine. Optimum reaction appears to be about pH 6·2–6·8, but the resistance to acidity is quite variable; B2 grows still at pH 4·3, and 11 stops growth at pH 5·6. Strains 18 and B2 grow well at 37°C., the others scantily. No phenomena of dissociation have been observed in this group.

CORYNEBACTERIUM LACTICUM (Orla-Jensen), n. comb.

Synonym: *Microbacterium lacticum* Orla-Jensen (1919).

This organism proved to be a morphologically and biologically typical corynebacterium; this is also indicated by Orla-Jensen's instructive microphotographs. The genus *Microbacterium* cannot possibly stand as defined by Orla-Jensen, since of its four species, one is a true *Proactinomyces* (Jensen, 1932), one—*flavum*—an organism on the border-line between *Mycobacterium* and *Corynebacterium*, and *lacticum* and *liquefaciens* doubtless corynebacteria. As pointed out under *Myc. flavum*, their faculty of producing lactic acid does not make them a separate genus.

Cor. lacticum is in many respects, except for its acid-formation, studied in detail by Orla-Jensen (1919) and Wittern (1932), and its lower temperature-optimum, very similar to *Cor. pseudodiphtheriticum*. Orla-Jensen states that it will show some proteolysis in milk, whereas Wittern found it non-proteolytic. The present strain was non-proteolytic, but *Cor. pseudodiphtheriticum* appeared faintly proteolytic in milk (Table II). With this last organism we have arrived at the group of the real "diphtheroids", mainly non-proteolytic organisms, requiring protein, and chiefly occurring as parasites in warm-blooded animals.

A Tentative Scheme for the Identification of Saprophytic Corynebacteria.

I. Organisms with pronounced morphological differentiation; slimy or myceloid variants common (*Cor. diphtheriae* and the corynebacteria of de Negri, Bergstrand and Mellon are probably of this group).
  A. Good growth in protein-free media.
    1. Cystites big and numerous in dextrose-agar.
      a. Cream-coloured to chrome-yellow growth on agar ..... *Cor. helvolum.*
      b. Blue insoluble pigment in dextrose-agar ..... *Cor. insidiosum.*
    2. Cystites less typical. Weaker proteolytic than (1) ..... *Cor. cremoides.*
  B. Very scant growth in protein-free media. Characteristic cystites in dextrose-agar ..... *Cor. tumescens.*

II. Organisms with generally less morphological differentiation, and little tendency to formation of variants. (Most "diphtheroids", e.g., the *pseudodiphtheriticum-* and *xerosis*-types, seem to belong here.)
  A. Good growth in protein-free media. Characteristic curved cells on asparagine-agar. Strongly proteolytic.
    1. Long sinuous filaments in protein media ..... *Cor. filamentosum.*
    2. Short straight rods in protein media ..... *Cor. simplex.*
  B. Scant growth, or none, in protein-free media. Feebly proteolytic or non-proteolytic.
    1. Pink growth on agar ..... *Cor. nubilum.*
    2. White to yellow growth on agar.
      a. Cellulose decomposed ..... *Cor. fimi.*
      b. Cellulose not decomposed.
        x. Viscid, deep ochre-yellow growth on potato ... *Cor. michiganense.*
        xx. Pasty, white to lemon-yellow growth.
          a. Gelatine is liquefied (mostly) .... *Cor. liquefaciens* and related group.

β. Gelatine is not liquefied .... *Cor. lacticum* and *pseudodiphtheriticum*-group. (Transition to "diphtheroids" proper.)

TABLE II.

Action of Corynebacteria in Milk. Incubation 28 days, 28°C.

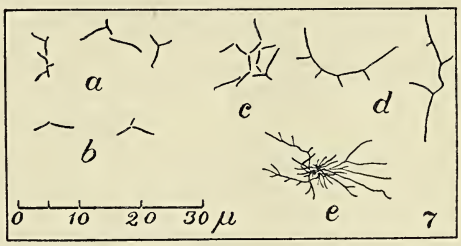
Organism.	Formol-titrating N, mgm.		Organism.	Formol-titrating N, mgm.	
	Per 10 c.c.	Excess over Control.		Per 10 c.c.	Excess over Control.
Cor. helvolum N3	16·7	13·6	*Cor. filamentosum* 163	5·7	2·6
,, ,, A1	17·0	13·9	,, ,, 272a ..	5·5	2·4
,, ,, A4	15·1	12·0	,, *simplex* 282	18·9	15·8
,, ,, N1	15·0	11·9	,, ,, B	12·8	9·4
,, ,, Ca3	14·9	11·8	,, *nubilum*	2·8	(−0·3)
,, ,, C	14·4	11·3	,, *tumescens* A	7·4	3·3
,, ,, 279	14·4	11·3	,, ,, 18	3·9	0·8
,, ,, 163S	14·3	11·2	,, *michiganense*, soil str. ..	9·0	5·9
,, ,, Ca1	14·1	11·0	,, *liquefaciens*	5·5	2·4
,, ,, Bb	13·7	10·6	*Liquef.*—group 276	3·5	0·4
,, ,, B	11·4	8·3	,, ,, 276, 8 w.[1] ..	8·0	4·9
,, ,, 163	11·1	8·0	,, ,, B2	3·2	(0·1)
,, ,, 121	7·2	4·1	,, ,, B2, 8 w.[1] ..	3·0	(−0·1)
,, *cremoides* C	5·8	2·7	,, ,, 11	3·3	(0·2)
,, ,, A	4·5	1·4	,, ,, 18	3·0	(−0·1)
,, *insidiosum*	6·2	3·1	,, ,, B3	4·0	0·9
,, *filamentosum* 279	15·7	12·6	*Cor. lacticum*	2·9	(−0·2)
,, ,, 272b	14·6	11·5	,, *pseudodiphtheriticum* I ..	4·6	1·5
,, ,, 276	13·5	10·4	,, ,, II ..	4·3	1·2

Control titrations as in Table I.
[1] Incubated for 8 weeks.

Organisms Uniting Corynebacteria and Proactinomycetes.

Two strains, A and M, isolated from garden soil and grass soil, respectively, were found to combine the characters of *Proactinomyces* and *Corynebacterium*. In dextrose-agar, Sabouraud's agar and potato they showed no extraordinary morphological features, appearing after one day at 18° to 30°C., both in stained preparations and by direct agar-microscopy, as fairly long, sometimes branching, slender rods in angular arrangement, mostly 0·3–0·5μ × 2–12μ (Pl. ii, fig. 48), A still thinner than M. No real mycelia are seen, and after 2–3 days they appear as short rods and minute cocci (Pl. ii, fig. 50). So far one would not hesitate to declare them corynebacteria. But in sugar-free nutrient agar they produce, after one day at low temperatures (17–18°C.) extensive, branching mycelia of a definite *Proactinomyces*-type (Text-fig. 7), although without any aerial growth. The mycelia remain for several days, but are so fragile as to be observable almost only by direct agar-microscopy; later they divide into rods and cocci. Strain M produces similar mycelia in dulcite-casein-agar and in milk-agar (Pl. ii, fig. 49); they divide rapidly into rods on the surface, but remain undivided for a considerable time in the depth of the agar. No mycelia are formed in liquid media. Strain A formed a "myceloid" variant after 208 days' growth in lithium-solution.

It appeared after one day at 28–30°C. as very long branching mycelia, in dextrose-agar changing into short rods and cocci after a few days, but in Sabouraud's agar remaining filamentous for several weeks, with oval to pear-shaped cystites of 1·0–1·2μ thickness.



Text-figure 7.—Organism between *Corynebacterium* and *Proactinomyces*, strain A. Direct agar-microscopy. *a-b*. Dextrose-agar, 20 hours, 28°C.; *c*. Twenty hours, 18°C.; *d*. Sugar-free nutrient agar, 20 hours, 17°C.; *e*. Two days; 17°C. (× 500).

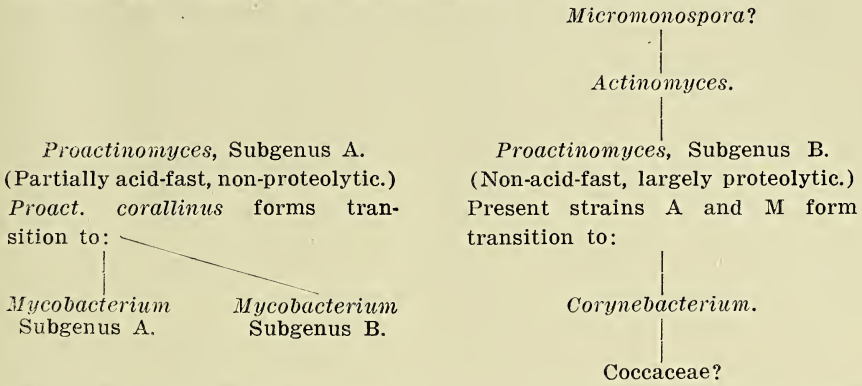
Culturally the two strains are somewhat similar to the *liquefaciens*-group: in asparagine-agar only very scant growth, in dextrose-agar and Sabouraud's agar fair to good growth, smooth and glistening, cream-coloured to chrome-yellow (especially strain A at 18–20°C.), in gelatine stab filiform to finely arborescent growth, very slow liquefaction. Gelatine-colonies of A are spherical and granular, but of M definitely myceloid. M coagulates milk and re-digests it slowly; A lost this faculty after a few transfers. M produces an abundant, dull yellow growth on milk-agar, the surface at first smooth and cartilaginous, after 4–8 days folded and soft, with strong clearing of the medium; the growth of A is slower, developing deep into the agar, with soft surface and hard deeper layer, very slow clearing. Non-protein sources of N are hardly utilized. M reduces nitrate to nitrite. A inverts saccharose. Diastatic action is good in M, faint in A. Both produce acid from arabinose, dextrose, levulose, galactose, maltose, saccharose, and lactose, M also from glycerine. Proteolytic action in milk is faint or absent. M is strongly haemolytic, and grows well at 37°C., A hardly at all. Optimal reaction pH 6·8–7·2. Growth stops at pH 5·3–5·6.

The two strains seem too dissimilar to be united into a single species, and have therefore been left unnamed. We have here a case where it is within the power of the experimenter to make the organisms appear either as typical corynebacteria or as typical proactinomycetes simply by altering the composition of the medium and/or the temperature of incubation. As to the question of their systematic position, one might be inclined to follow the principle of Enderlein (1925) according to which the stage of highest morphological differentiation (the "culminant") indicates the genus, and thus to regard them as members of *Proactinomyces*, closely related to *Proact. mesentericus* (Jensen, 1932; cf. also Wittern, 1932). But it is to be remembered that a logical adherence to this principle would entail the transferring to *Proactinomyces* of *Cor. helvolum* and indeed of any corynebacterium in which myceloid variants might be found.

Relation of Corynebacterium *and* Mycobacterium *to Other Groups of Microorganisms.*

The mycobacteria and the corynebacteria make two natural groups, which, however, are not sharply separated from each other. Subgenus B of the

mycobacteria stands, particularly as represented by *Myc. flavum*, closely midway, and each of the genera is closely connected with the genus *Proactinomyces*, from which both may be derived, as shown below:



Thus the genera *Mycobacterium*, *Corynebacterium*, *Proactinomyces*, *Actinomyces* and *Micromonospora* form a natural and consistent group of microorganisms—the order Actinomycetales—whose natural kinship is proved by the existence of transitional forms between all subgroups. Firstly, the mycobacteria of subgenus B are simply proactinomycetes of subgenus A, which have lost their mycelial growth, wholly or nearly; a certain "reversion" to this mode of growth is represented by the pink variants of *Myc. rubropertinctum*. The fact that such variants arise in single-cell cultures speaks for the correctness of the alleged existence of similar variants in *Myc. tuberculosis* (see introduction). This suggests a connection between *Mycobacterium*, subgenus A, and *Proactinomyces*, subgenus A, a point also supported by the ability of mycobacteria to assume an *Actinomyces*-like growth in the animal body (Schulze, 1899; Abbott and Gildersleeve, 1902, *et al.*). The relationship between *Mycobacterium* A and *Proactinomyces A* is further demonstrated by the mode of cell division (which has become more distinctly slipping in the former), the acid-fastness, the existence of rudimentary aerial "mycelium", and the biochemical similarities. Between the subgenera A and B of *Proactinomyces* the partially acid-fast *Proact. polychromogenus* and *minimus* seem in a certain way to form a connection, since they produce some proteolysis in milk (Table I). The connection between *Corynebacterium* and *Proactinomyces* subgenus B is obvious: the transition proceeds from the long-hyphed *Proact. flavescens* (which again forms the transition to *Actinomyces*; Jensen, 1932) over *Proact. actinomorphus* and *mesentericus* to the above-mentioned two strains A and M, which appear as either proactinomycetes or corynebacteria according to circumstances. An approach to the *Proactinomyces*-type from the other side is exemplified by the myceloid variants of *Cor. helvolum*, which lend some support to the allegation of actinomyces-like types of *Cor. diphtheriae*, as mentioned in the introduction. A variation in the opposite direction seems represented by a phenomenon reported by Wittern (1932): stabilization of a small rod-shaped to coccoid variant of *Proact. mesentericus*.*

There are certain other groups of microorganisms which doubtless stand in close relationship to the corynebacteria, such as the so-called propionic acid

* The strain in my possession has not shown this phenomenon.

bacteria ("*Propionibacter*"). Troili-Petersson (1909) first called attention to their morphological resemblance to the diphtheria bacillus, and van Niel (1928) classified them as a genus closely related to the corynebacteria, distinguished from these by their microaerophilic character and their obligate fermentative metabolism. It is to be remembered in this connection that microaerophilic corynebacteria have also been described (Eberson, 1918; Thomson and Thomson, 1926; Steck, 1932), but their metabolic properties have not been studied; such a study might possibly show that there is no sharp limit at all between *Corynebacterium* and *Propionibacter*. The lactic acid bacilli ("*Lactobacillus*" or "*Plocamobacter*") are considered close relations of the corynebacteria by Lehmann and Neumann (1927) and van Niel (1928). Among other microaerophilic and anaerobic bacteria which seem related to the corynebacteria, we might also mention the "*Bac.*" *cornutus* and "*Diplobac.*" *acuminatus* Distaso (1912) and an organism described by Davis and Mattick (1930).

While all these organisms seem naturally related, a more doubtful point confronts us in the relation between corynebacteria and true cocci. Stabilization of cocci from corynebacteria was alleged by Mellon (1917) and Kuschnarjew (1930). Novak and Henrici (1933) obtained from a typical chromogenic *Actinomyces* an organism which on plain agar appeared as a staphylococcus, but which in dextrose-agar formed rods and branching filaments (cf. *Cor. helvolum.*). Ohlmacher (1902), Kermogant (1922) and Mellon (1926) have mentioned a diphtheroid-like appearance of streptococci under certain conditions. Thomson and Thomson (1926), and Jensen and Morton (1931) describe organisms appearing either as diphtheroids or as streptococci, according to the medium. Prissick (1933) claims to have transformed a streptococcus into a diphtheroid and back again. Since, as Thomson and Thomson (1926) point out, delayed cell division may cause true cocci to appear like diphtheroids,* and since the warnings of Klieneberger (1932) ought also to be kept in mind, it would for the present seem advisable to regard the phylogenetic connection between corynebacteria and cocci as nothing more than a possibility, or, as Thomson and Thomson (1926, pp. 139–140) express it: "It is not beyond the range of possibility that there may be some organisms which form a connecting line between the streptococci and the diphtheroids. . . . It is just possible . . . that some of them may find a place in a separate genus."

The question which groups represent the ancestral and which the descendant forms can at present hardly be made the subject of more than hypothetical speculation, although one might be tempted to regard the formation of longer, branched cells in the young stages as evidence of descent from mycelial forms (cf. Henrici, 1928). This idea might suggest *Proactinomyces* as a primitive group, giving rise on one side by further differentiation to *Actinomyces* and *Micromonospora*, and on the other by reduction to *Mycobacterium* and *Corynebacterium*, and from the latter possibly to the true cocci. But here we are, as Henrici emphasizes, on purely hypothetical ground.

Finally we must mention Kuhn's *Pettenkoferia*-theory in its bearings upon the phenomena of variation in these organisms. According to this theory (Kuhn and Sternberg, 1931) all bacteria live in symbiosis with certain protozoa-like

* This may account for the diphtheroid-like appearance of certain micrococci in saline media (Matzuschita, 1900).

organisms, the "*Pettenkoferia*", possessing a definite life-cycle in which the bacteriophage represents a stage. Bacteria are supposed to occur only in two main forms, rods (without true branching) and cocci, and all so-called phenomena of pleomorphism are claimed to be due to the influence of parasitizing *Pettenkoferia*, which cause an abundant slime-production in cultures rich in them. Although I do not venture to pass judgment on the *Pettenkoferia*-theory generally, the phenomena observed in the mycobacteria and corynebacteria seem explicable without it, mainly on the basis of the cytomorphosis-theory of Henrici (1928). That true branching occurs in these organisms is easily seen by following the growth directly under the microscope, and the conclusion of Kuhn and Sternberg, that there is no relationship between the tubercle bacillus and the actinomycetes, cannot possibly be agreed to. Also the fact that swollen cystites (which according to Kuhn and Sternberg are infected with *Pettenkoferia*) may develop into apparently normal colonies (cf. Klieneberger, 1930), speaks against the theory, but not absolutely, since Kuhn and Sternberg say that the *Pettenkoferia* are often concealed and hard to detect. A stronger point against the theory is represented by the slimy variants of *Cor. helvolum* and related species; according to the theory these should be rich in *Pettenkoferia* and therefore likely to show phenomena of bacteriophagy, but no "taches vièrges" or other signs hereof have been observed. Neither did such phenomena appear in other cultures which had passed through lithium-chloride media (cf. Klieneberger, 1930).

Summary.

A morphological and biological study has been made of a number of soil mycobacteria and corynebacteria. The genus *Mycobacterium* is comparatively rare in soil. It has two subgenera, A and B. The former represents the mycobacteria proper, with *Myc. tuberculosis* as type-species. These organisms show a pronounced slipping growth, are strongly acid-fast, and produce little or no acid from carbon compounds. Subgenus B has *Myc. coeliacum* as type-species. These organisms show a characteristic cytomorphosis consisting in a transformation from long, often branching rods into cocci. They are only weakly acid-fast, and have more tendency to acid-production than those of subgenus A. The whole genus attaches itself closely to the partially acid-fast, non-proteolytic proactinomycetes. Most saprophytic mycobacteria are capable of decomposing paraffine, and all show a characteristic growth in milk, but no proteolytic or diastatic properties. A dissociation into "plane" and "perrugose" types is common among them, particularly under the influence of acid reaction.

The genus *Corynebacterium* is common in the soil. The saprophytic corynebacteria are not acid-fast, do not attack paraffine, but are mostly proteolytic, often diastatic, and generally capable of producing acid from numerous carbon compounds; they appear in no way sharply distinguished from the parasitic "diphtheroids". They show a wide range of morphological variation, and some of them produce variants with a strong slime formation, generally connected with a loss of formation of swollen cells, or "myceloid" variants which come near to the *Proactinomyces*-type. Such variants arise mainly in old cultures under the influence of lithium or uranium-salts. There is less evidence of "smooth" and "rough" variants. The phenomena of morphological variation seem more naturally explicable according to the cytomorphosis-theory of Henrici than according to the life-cycle theories of Löhnis and Enderlein. Only one species (*Cor. tumescens*)

showed some evidence of reproduction by gonidia. The *Pettenkoferia*-theory of Kuhn did not find any positive support in the results obtained with these organisms.

Numerous previously-described bacteria seem to belong to *Mycobacterium* subgenus B, or to *Corynebacterium*. Three new species (*Cor. tumescens, filamentosum*, and *simplex*) are described, as well as eight new combinations, with synonyms. Organisms have been found, which form a natural transition between *Corynebacterium* and the non-acid-fast, proteolytic group of *Proactinomyces*.

Cultures of the new species and other characteristic groups have been forwarded to The National Collection of Type Cultures, Lister Institute of Preventive Medicine, London.

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EXPLANATION OF PLATES I-II.

Photographs represent nigrosine-preparations, or films stained with dilute carbol-fuchsin, magnification × 750, unless otherwise stated.

Plate i.

Figs. 1-5.—*Myc. coeliacum*, AIII, living objects, × 350. 1-2, on dextrose-agar, 1 day, room temp., same organism with interval of 2 hours; 3-5, on glycerine-agar, 1 day, room temp., same organisms with intervals of 2 hours.

Fig. 6.—Same, asparagine-agar, 2 days, 28°C.

Fig. 7.—*Myc. coeliacum*, authentic, egg medium, 4 days, 28°C.

Fig. 8.—*Myc. rubropertinctum*, 279, asparagine-agar, 1 day, 28°C.

Fig. 9.—*Myc. equi*, M, dextrose-agar, 2 days, 28°C.

Fig. 10.—*Myc. flavum*, dextrose-agar, 3 days, 28°C.

Figs. 11-25.—*Cor. helvolum*. 11-13, strains Ca1, 163, and 279, dextrose-agar, 1 day, 28-30°C.; 14-15, strains A4 and C, potato, 1 day, 28°C.; 16, str. 163, nutrient agar, 3 days, 30°C.; 17, same, saccharose-nitrate-agar, 6 days, 28°C.; 18, str. C, asparagine agar, 2 days, 30°C.; 19, A4, dextrose-agar, 5 days, 28°C.; 20, N3, dextrose-agar, 6 days, 30°C.; 21, same, 6-days-old cystites germinating after 20 hours, room temp., on casein-agar; 22, 163, potato, 2 days, 37°C.; 23, C, potato, 4 days, 37°C.; 24, myceloid variant of N3, potato, 1 day, 30°C.; 25, myceloid variant of Bb, potato, 1 day, 30°C.

Plate ii.

Figs. 26-28.—*Cor. helvolum*, myceloid variant of str. 163, dextrose-agar. 26, 5 days, 28°C. (slope); 27, 6 days, 28°C. (condensation-water); 28, 16 days, 28-30°C. (slope).

Figs. 29-31.—*Cor. filamentosum*. 29, 272*b*, asparagine-agar, 2 days, 18°C.; 30, 276, dextrose-agar, 1 day, 28°C.; 31, 279, asparagine-agar, 5 days, 28°C.

Figs. 32-34.—*Cor. simplex*. 32, B, asparagine-agar, 1 day, 28°C.; 33, 282, dextrose-agar, 1 day, 28°C.; 34, B, dwarf-variant, asparagine-agar, 1 day, 28°C.

Fig. 35.—*Cor. michiganense*, soil strain, dextrose-agar, 1 day, 37°C.

Figs. 36-44.—*Cor. tumescens*. 36, A, dextrose-agar, 1 day, 28°C. (× 562); 37, B, Sabouraud's agar, 12 days, 16°C.; 38, B, Sabouraud's agar, 18 days, 28°C.; 39, A, transferred from dextrose-agar, 6 days, 28°C. to dextrose-agar, 20 hours, room temp., gonidia? (× 1125); 40, B, milk-agar, 8 days, 30°C.; 41, cystites from previous, germinating on Sabouraud's agar after 20 hours, 28°C.; 42, A, casein-agar, 1 day, 28°C. (× 562); 43, same, 3 days, 28°C. (× 562); 44, B, slimy variant, Sabouraud's agar, 5 days, 30°C.

Figs. 45-46.—*Cor. liquefaciens*-group, strain 276, dextrose-agar, 1 day and 3 days, 28°C.

Fig. 47.—*Cor. fimi*, dextrose-agar, 1 day, 28°C.

Fig. 48.—*Proactinomyces*-like organism, strain M, dextrose-agar, 1 day, 28°C.

Fig. 49.—Same, milk-agar, 1 day, 28°C.

Fig. 50.—Strain A, dextrose-agar, 2 days, 28°C.